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BIOASSAY IN WATER QUALITY ANALYSIS AND EFFLUENT MONITORING

This course is designed for those who need a practical competence in the design and use of bioassay and biomonitoring.

ENVIRONMENTAL PROTECTION AGENCY
Office of Water Program Operations
TRAINING PROGRAM

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INTRODUCTION

The biota of a natural aquatic ecosystem is the result of evolutionary processes in the course of which a delicate balance and complex interactions were established among various kinds of organisms and between those organisms and their environment. Some species can live in a wide range of environmental conditions and are found in many different systems throughout the world. Other species are restricted and their distribution is limited to certain habitats or in some cases to only one. Frequently, it is the latter group of species that have been most useful to man. Minor changes in their environments, especially if such changes are rapid, may upset the ecological balance and endanger the species.

Man has the ability to alter—to impair or improve—his environment and that of other organisms. His use of water to dispose of wastes of a technological society and his other alterations of aquatic environments have degraded his water resources. Water pollutants may alter natural conditions by reducing the dissolved oxygen content, by changing the temperature, or by direct toxic action that can be lethal or, more subtly, can affect the behavior, reproduction, and physiology of the organisms. Although a substance may not directly affect a species, it may endanger its continued existence by eliminating essential sources of food and metabolites. Furthermore, conditions permitting the survival of a given organism at one stage of its life may be intolerable at another stage.

This Section evaluates criteria and proposes recommendations that reflect scientific understanding of the relationships between freshwater aquatic organisms and their environment. Anything added to or removed from natural waters will cause some change in the system. For each use of water there are certain water quality characteristics that should be met to ensure the suitability of the water for that use.

The following general recommendations apply to a wide variety of receiving systems and pollutants:

- More stringent methods of control or treatment, or both, of waste inputs and land drainage should be applied to improve water quality as the demand for use increases.
- In recognition of the limitations of water quality management programs, consideration should be given to providing reserve capacity of receiving waters for future use.
- Bioassays and other appropriate tests, including field studies, should be made to obtain scientific evidence on the effect of wastewater discharges on the environment. Test procedures are recommended in this report.
- A survey of the receiving system to assess the impact of waste discharges on the biological community should be made on a regular basis, particularly prior to new discharges. Such surveys especially should cover the seasons most critical to the biological community. Background laboratory data should include bioassays using important local aquatic organisms and associated receiving waters. In addition to the more comprehensive surveys, some form of biomonitoring in the receiving system should be carried out routinely. A suggested list of ecological considerations is included in the section on Biological Monitoring.
- One of the principal goals is to insure the maintenance of the biological community typical of that particular locale or, if a perturbed community exists, to upgrade the receiving system to a quality which will permit reestablishment of that community.

COMMUNITY STRUCTURE AND PROTECTION OF SIGNIFICANT SPECIES

The natural aquatic environment includes many kinds of plants and animals that vary in their life history and in their chemical and physical requirements. These organisms are interrelated in many ways to form communities. Aquatic environments are protected out of recreational and scientific interest, for aesthetic enjoyment, and to maintain certain organisms of special significance as a source of food. There are two schools of thought as to how this can be accomplished. One is to protect the significant species, the assumption being that by so doing, the entire system is protected. The other approach is to protect the aquatic community, the assumption being that the significant species are not protected unless the entire system is maintained.

Community Structure

Because chemical and physical environments are continually changing—sometimes gradually and sometimes catastrophically—many species are necessary to keep the aquatic ecosystems functioning by filling habitats vacated because of the disappearance of other species. Likewise, when one kind of organism becomes extremely abundant because of the disappearance of one or more species, predator species must be available to feed on the overabundant species and keep it from destroying the functioning of the community. In a balanced ecosystem, large populations of a single species rarely maintain themselves over a long time because predators quickly reduce their number.

Therefore, the diverse characteristics of a habitat are necessary to the maintenance of a functioning ecosystem in the process of evolution. In the fossil record are found many species that were more common at one time than they are today and others that have been replaced entirely. If it were not for diverse gene pools, such evolutionary replacement would not have been possible.

Some aquatic environments present unusual extremes in their chemical and physical characteristics. They support highly specialized species that function as ecosystems in which energy flows and materials cycle. If these species are not present and functioning in this manner, such areas may become aesthetically distasteful, as has occurred for example in the alkaline flats of the West and the acid bogs of the Northeast, Midwest, and East.

Rare habitats support rare organisms that become extinct or endangered species if their habitats are impaired or eliminated. In the aquatic world there are many species of algae, fish, and invertebrates that are maintained only in such rare, fragile habitats. Man must understand them if he is to appreciate the process of evolution and the trend of ecological change that brings about drastic alterations to fauna and flora.

Protection of Significant Aquatic Species

An essential objective of freshwater quality recommendations is the protection of fish and other aquatic organisms for sport or commercial harvesting. This does not imply that all other aquatic species will be subject to potential extinction, or that an unaltered environment is the goal to be attained in all cases. The average person is usually interested in only a small number of aquatic species, principally fish; but it remains necessary to preserve, in certain unique or rare areas, a diversified environment both for scientific study and for maintaining species variety.

It is sometimes difficult to justify protection of isolated organisms not used by man unless it can be documented that they are ultimately essential to the production of desirable biota. In some instances it may be that a critical, sensitive species, irreplaceable in the food web of another more important species, is one known only to the biologist. In such instances, protection of the "less important" sensitive species could justifiably determine the water quality recommendation.

Because no single recommendation can protect all important sport and commercial species unless the most sensitive is protected, a number of species must be considered. The most sensitive species provide a good estimate of the range of sensitivity of all species.

ASSIMILATIVE CAPACITY OF FRESHWATER RECEIVING SYSTEMS

Waste discharges do not just go into water but rather into aquatic ecosystems. The capacity of such a system to receive and assimilate waste is determined by the physical, chemical, and biological interactions within the system. Thus the response is a function of the characteristics of both the ecosystem and the nature and quantity of the waste. Understanding the unique characteristics of each ecosystem will enable wise users to develop means to obtain maximum beneficial use with minimal damage to the system. Each aquatic ecosystem is sufficiently unique to require professional ecological advice to define the problems associated with waste discharge into a particular ecosystem. Such a procedure has not been customary in the past, and this has led to some unfortunate consequences, but the practice is becoming increasingly prevalent.

Aquatic systems receive from natural and man-made sources a variety of organic and inorganic materials. These materials through physical, chemical, and biological interaction are transported, rendered, converted, respired, incorporated, excreted, deposited and thus assimilated by the system. However, not all systems can receive and assimilate the same quantity or kinds of waste materials. The capacity of each system to transform waste without damage to the system is a function of the complexity of environmental factors.

Physical factors such as flow velocity, volume of water, bottom contour, rate of water exchange, currents, depth, light penetration, and temperature, govern in part the ability of a system to receive and assimilate waste materials. This ability is a function of the reaeration capability of the system, the physical rendering of wastes, and other physical, chemical, and biological factors. Most flowing systems have a greater reaeration capacity than standing waters. Furthermore, flowing systems are open systems with continual renewal of water, whereas standing waters are closed systems and act as traps for pollutants.

Temperature plays a vital role in the rate of chemical reactions and the nature of biological activities in freshwater and in governing the receiving and assimilative capacity of a system. Most temperate lakes are thermally stratified part of the year, except when there are small differences between surface and bottom temperatures in the spring and fall. As a consequence little exchange occurs between layers during the period of stratification. In organically enriched lakes and reservoirs, depletion of soluble oxygen typically occurs in the bottom layer because there is little or no photosynthesis and little mixing with the oxygen-rich surface layer. As a result, substances are released from the sediments because certain compounds have a much greater solubility in a reduced state.

The unique chemical characteristics of water govern in part the kinds and quantities of waste a system may receive. Some of the important chemical characteristics are hardness, alkalinity, pH (associated with the buffering capacity), and nutrients such as carbon, nitrogen, and phosphorus. Because of synergistic or antagonistic interaction with receiving water, the effects of a waste on a wide variety of receiving systems are hard to predict.

MIXING ZONES

When a liquid discharge is made to a receiving system, a zone of mixing is created. Although recent public, administrative, and scientific emphasis has focused on mixing zones for the dispersion of heated discharges, liquid wastes of all types are included in the following considerations. (For a further discussion of Mixing Zones see Appendix II-A.)

DEFINITION OF A MIXING ZONE

A mixing zone is a region in which a discharge of quality characteristics different from those of the receiving water is in transit and progressively diluted from the source to the receiving system. In this region water quality characteristics necessary for the protection of aquatic life are based on time-exposure relationships of organisms. The boundary of a mixing zone is where the organism response is no longer time-dependent. At that boundary, receiving system water quality characteristics based on long-term exposure will protect aquatic life.

Recommendation

Although water quality characteristics in mixing zones may differ from those in receiving systems, to protect uses in both regions it is recommended that mixing zones be free of substances attributable to discharges or wastes as follows:

- **materials which form objectionable deposits;**
- **scum, oil and floating debris;**
- **substances producing objectionable color, odor, taste, or turbidity;**
- **conditions which produce objectionable growth of nuisance plants and animals.**

GENERAL PHYSICAL CONSIDERATIONS

The mass emission rates of the most critical constituents and their relationship to the recommended values of the material in the receiving water body are normally the primary factors determining the system-degradation potential of an effluent. Prior to establishment of a mixing zone the factors described in Waste Capacity of Receiving Waters (Section IV, pp. 228–232) and Assimilative Capacity (This Section, p. 111) should be considered and a decision made on whether the system can assimilate the discharge without damage to beneficial uses. Necessary data bases may include:

- Discharge considerations—flow regime, volume, design, location, rate of mixing and dilution, plume behavior and mass-emission rates of constituents including knowledge of their persistence, toxicity, and chemical or physical behavior with time.
- Receiving system considerations—water quality, local meteorology, flow regime (including low-flow records), magnitude of water exchange at point of discharge, stratification phenomena, waste capacity of the receiving system including retention time, turbulence and speed of flow as factors affecting rate of mixing and passage of entrained or migrating organisms, and morphology of the receiving system as related to plume behavior, and biological phenomena.

Mathematical models based in part on the above considerations are available for a variety of ecosystems and discharges. (See Appendix II-A.) All such mathematical models must be applied with care to each particular discharge and the local situation.

Recommendation

To avoid potential biological damage or interference with other uses of the receiving system it is recommended that mixing zone characteristics be defined on a case-by-case basis after determination that the assimilative capacity of the receiving system can safely accommodate the discharge taking into consideration the physical, chemical, and biological characteristics of the discharge and the receiving system, the life history and behavior of organisms in the receiving system, and desired uses of the waters.

GENERAL BIOLOGICAL CONSIDERATIONS

Organisms in the water body may be divided into two groups from the standpoint of protection within mixing zones: (1) nonmobile benthic or sessile organisms; (2) weak and strong swimmers.

1. Nonmobile benthic or sessile organisms in mixing zones may experience long or intermittent exposures exceeding recommended values for receiving systems and therefore their populations may be damaged or eliminated in the local region. Minimum damage to these organisms is attained by minimizing exposure of the bottom area to concentrations exceeding levels resulting in harm to these organisms from long-term exposure. This may be accomplished by discharge location and design.

The mixing zone may represent a living space denied the subject organisms and this space may or may not be of significance to the biological community of the receiving system. When planning mixing zones, a decision should be made in each case whether the nonmobile benthic and sessile organisms are to be protected.

Recommendation

To protect populations of nonmobile benthic and sessile organisms in mixing zones it is recommended that the area of their habitat exposed to water quality poorer than recommended receiving system quality be minimized by discharge location and design or that intermittent time-exposure history relationships be defined for the organisms' well-being.

2. Biological considerations to protect planktonic and swimming organisms are related to the time exposure history to which critical organisms are subjected as they are carried or move through a mixing zone. The integrated time exposure history must not cause deleterious effects, including post-exposure effects. In populations of important species, effects of total time exposure must not be deleterious either during or after exposure.

Weak swimmers and drifting organisms may be entrained into discharge plumes and carried through a mixing zone. In determining the time exposure history and responses of the organisms, the possibility of delayed effects, such as death, disease, and increased vulnerability to predation, should be investigated.

Strong swimmers are capable of moving out of, staying out of, or remaining in a mixing zone. Water quality characteristics which protect drifting organisms should also protect migrating fish moving through mixing zones. However, there are some discharges that attract animals into discharge channels and mixing zones where they are vulnerable to death or shock due to short-term changes in water quality, such as rapid temperature fluctuations. This vulnerability should be recognized and occurrences that expose it should be guarded against (see Chlorine, page 189).

Some free-swimming species may avoid mixing zones and as a consequence the reduced living space may limit the population.

Free-swimming species may be attracted to a discharge. Chronic low-level exposure to toxicants may cause death or affect growth, reproduction or migratory instincts, or result in excessive body-burdens of toxicants hazardous for human consumption.

Recommendation

To protect drifting and both weak and strong swimming organisms in mixing zones it is recommended that scientifically valid data be developed to demonstrate that the organisms can survive without irreversible damage, the integrated time-exposure history to be based on maximum expected residence time so that deleterious effects on populations of important species do not occur.

MEETING THE RECOMMENDATIONS

In mixing zones the exposure of organisms to stress is of greater intensity but usually of shorter duration than in the receiving waters, assuming no attraction by the discharge. The objective of mixing zone water quality recommendations is to provide time exposure histories which produce negligible or no effects on populations of critical species in the receiving system. This objective can be met by: (a) determination of the pattern of exposure in terms of time and concentration in the mixing zone due either to activities of the organisms, discharge schedule, or currents affecting dispersion; and (b) determination that delayed effects do not occur.

Protection would be achieved if the time of exposure met the relationship $T/ET(x) \leq 1$ where T is the time of the organism's exposure in the mixing zone to a specified concentration, and ET(x) is the effective time of exposure to the specified concentration, C, which produces (x) per cent response in a sample of the organisms, including delayed effects after extended observation. The per cent response, (x), is selected on the basis of what is considered negligible effects on the total population and is then symbolized ET(25), ET(5), ET(0.1), etc.

Because concentrations vary within mixing zones, a more suitable quantitative statement than the simple relationship $T/ET(x) \leq 1$ is:

$$\frac{T_1}{ET(x) \text{ at } C_1} + \frac{T_2}{ET(x) \text{ at } C_2} + \frac{T_3}{ET(x) \text{ at } C_3} \cdots + \frac{T_n}{ET(x) \text{ at } C_n} \leq 1$$

where the time of exposure of an organism passing through the mixing zone has been broken into increments, T_1, T_2, T_3, etc. The organism is considered to be exposed to concentration C_1 during the time interval T_1, to concentration C_2 during the time interval T_2, etc. The sum of the individual ratios must then not exceed unity. (See caveat below, Short Time Exposure Safety Factors.)

Techniques for securing the above information, application to a hypothetical field situation, comments, caveats, and limitations are expressed in Appendix II–A, Mixing Zones, Development of Integrated Time Exposure Data, p. 403. Tabular data and formulae for summation of short-term effects of heated discharges on aquatic life are provided in the Heat and Temperature discussion, page 151.

SHORT TIME EXPOSURE SAFETY FACTORS

This concept of summation of short-term effects and extrapolation is an approach which tests the applicability of present bioassay methodology and precision and may not be universally applicable to all types of discharges. Conservatism in application should be practiced. When developing the summation of short-term thermal effects data, a safety factor of two degrees centigrade is incorporated. In development of summation of short-term toxicity effects data, a safety factor exists if a conservative physiological or behavioral response is used with effective time of exposure. However, when mortality is the response plotted, an application factor must be incorporated to provide an adequate margin of safety. This factor can most easily be applied by lowering the sum of the additive effects to some fraction of 1 so that the sum of $T_1/(ET(x)$ at $C_1) \cdots +$ $T_n/(ET(x)$ at $C_n)$ then equals 0.9, or less. The value must be based on scientific knowledge of the organism's behavior and response to the contaminants involved.

Recommendation

When developing summation of short-term exposure effects it is recommended that safety factors, application factors, or conservative physiological or behavioral responses be incorporated into the bioassay or extrapolation procedures to provide an adequate margin of safety.

OVERLAPPING MIXING ZONES

If mixing zones are contiguous or overlap, the formula expressing the integrated time exposure history for single plumes should be adjusted. Synergistic effects should be investigated, and if not found, the assumption may be made that effects of multiple plumes are additive.

Recommendation

When two plumes are contiguous or overlap and synergistic effects do not occur, protection for aquatic life should be provided if the sum of the fractions of integrated time exposure effects for each plume total ≤ 0.5. Alternatively, protection should be provided if the sum of the fractions for both plumes (or more than two contiguous or overlapping plumes) is ≤ 1. (See caveat above, Short Time Exposure Safety Factors.)

INTERIM GUIDELINE

In the event information on summation effects of the integrated time exposure history cannot be satisfactorily provided, a conservative single figure concentration can be used for all parts of the mixing zone until more detailed determinations of the time-exposure relationships are developed. This single, time-dependent median lethal concentration should be subject to the caveats found throughout this Section and Appendix II–A regarding delayed effects and behavioral modifications. Because of the variables involved, the single value must be applied in the light of local conditions. For one situation a 24-hour LC50 might be adequate to protect aquatic life. In another situation a 96-hour LC50 might provide inadequate protection.

CONFIGURATION AND LOCATION OF MIXING ZONES

The time-dependent three dimensional shape of a discharge plume varies with a multitude of receiving system physical factors and the discharge design. While time exposure water quality characteristics within mixing zones are designed to protect aquatic life, thoughtful placement of the discharge and planned control of plume behavior may increase the level of ecosystem protection, e.g., floating the plume on the surface to protect the deep water of a channel; discharging in midstream or offshore to protect biologically-important littoral areas; piping the effluent across a river to discharge on the far side because fish historically migrate on the near side; or piping the discharge away from a stream mouth which is used by migrating species. Such engineering modifications can sometimes accomplish what is necessary to meet biological requirements.

Onshore discharges generally have more potential for interference with other uses than offshore discharges. For example the plume is more liable to impinge on the bottom in shallow areas of biological productivity and be closer to swimming and recreation areas.

PROPORTIONAL RELATIONSHIP OF MIXING ZONES TO RECEIVING SYSTEMS

Recommendations for mixing zones do not protect against the long-term biological effects of sublethal conditions. Thus water quality requirements necessary to protect all life stages and necessary functions of aquatic organisms such as spawning and larval development, are not provided in mixing zones, and it is essential to insure that adequate portions of every water body are free of mixing zones. The decision as to what portion and areas must be retained at receiving water quality values is both a social and scientific decision. In reaching this decision, data input should include current and projected information on types and locations of intakes and discharges; percentage of shoreline necessary to provide adequate spawning, nursery, and feeding areas; and other desired uses of the water.

Recommendation

It is recommended that the total area or volume of a receiving system assigned to mixing zones be limited to that which will: (1) not interfere with biological communities or populations of important species to a degree which is damaging to the ecosystem; (2) not diminish other beneficial uses disproportionately.

ZONES OF PASSAGE

In river systems, reservoirs, lakes, estuaries, and coastal waters, zones of passage are continuous water routes of such volume, area, and quality as to allow passage of free-swimming and drifting organisms so that no significant effects are produced on their populations.

Transport of a variety of organisms in river water and by tidal movements in estuaries is biologically important in a number of ways; e.g., food is carried to the sessile filter feeders and other nonmobile organisms; spatial distribution of organisms and reinforcement of depauperate populations is enhanced; embryos and larvae of some fish species develop while drifting. Anadromous and catadromous species must be able to reach suitable spawning areas. Their young (and in some cases the adults) must be assured a return route to their growing and living areas. Many species make migrations for spawning and other purposes. Barriers or blocks which prevent or interfere with these types of essential transport and movement can be created by water of inadequate chemical or physical quality.

Water quality in the zone of passage should be such that biological responses to the water quality characteristics of the mixing zone are no longer time-dependent (see Definition of Mixing Zone on page 112). However, where a zone of passage is to be provided, bioassays determining time-exposure responses in the mixing zone should include additional requirements to assess organism behavior. In the mixing zone discussion above it is assumed that entrainment in the plume will be involuntary. However, if there is attraction due to plume composition, exposure in the plume could be very much longer than would be predicted by physical modeling. If avoidance reactions occur, migration may be thwarted. Thus, concentrations in both the mixing zone and the zone of passage should be reduced before discharge to levels below those at which such behavioral modifications affect the populations of the subject organisms.

Modern techniques of waste water injection such as diffusers and high velocity jets may form barriers to free passage due to responses of organisms to currents. Turbulence of flows opposing stream direction may create traps for those organisms which migrate upstream by orientation to opposing currents. These organisms may remain in the mixing zone in response to currents created by the discharge.

Recommendation

Because of varying local physical and chemical conditions and biological phenomena, no single-value recommendation can be made on the percentage of river width necessary to allow passage of critical free-swimming and drifting organisms so that negligible or no effects are produced on their populations. As a guideline no more than ⅔ the width of a water-body should be devoted to mixing zones thus leaving at least ⅓ free as a zone of passage.

BIOLOGICAL MONITORING

Monitoring of aquatic environments has traditionally included obtaining physical and chemical data that are used to evaluate the effects of pollutants on living organisms. Biological monitoring has received less emphasis than chemical or physical monitoring, because biological assessments were once not as readily amenable to numerical expression and tended to be more time consuming and more expensive. This is no longer true. Aquatic organisms can serve as natural monitors of environmental quality and should be included in programs designed to provide continuous records of water quality, because they integrate all of the stresses placed on an aquatic system and reflect the combined effect. Chemical–physical assessments identify individual components, so the two types of assessments are mutually supporting rather than mutually exclusive.

A biological monitoring program is essential in determining the synergistic or antagonistic interactions of components of waste discharges and the resulting effects on living organisms. However, biological monitoring does not replace chemical and physical monitoring; each program provides information supplemental to the others.

PROGRAMS

An ideal biological monitoring program has four components: (1) field surveys, (2) in-plant biological monitoring, (3) bioassays, and (4) simulation techniques. Obviously no biological monitoring program is routine, nor does it necessarily have to include all of the above components. However, each of the components provides valuable and useful information.

FIELD SURVEYS

Field surveys are needed to obtain adequate data on biological, chemical, and physical water quality to determine the nature of the system and the possible adverse effects of waste discharges on beneficial uses of the system. Two methods for continuously monitoring the effects of pollution on a receiving water have been described. Patrick et al. (1954)[6]* described the use of diatoms as natural monitors of various types of pollution. Various species of shellfish, especially oysters suspended in trays, have been described as an effective method of monitoring pollution (Galtsoff et al. 1947).[4] Field surveys should be carried out at suitable intervals depending on local conditions. For example, in determining the impact of a new or relocated municipal or industrial discharge, it is desirable to perform the following functions:

- survey the stream as a part of the site selection procedure;
- continue the field survey prior to construction to determine existing water quality: at this time it is also useful to make bioassays using simulated plant wastes and representative organisms from the receiving systems, and to establish biomonitoring stations;
- monitor the effects of construction;
- carry out bioassays using actual plant wastes and effluents after the plant is in operation, and make field surveys to determine any changes from preconstruction results.

BODY BURDENS OF TOXICANTS

Body burdens of toxicants that can be concentrated by biota should be measured regularly. These data can provide early warning before concentrations in water become readily available and can provide warnings of incipient effects in the biota being monitored.

IN-PLANT BIOLOGICAL MONITORING

Present information systems do not provide data rapidly enough to be of use in environmental management, because the constituents of a waste stream are likely to vary from hour to hour and from day to day. Potentially harmful materials should be detected before they enter the receiving water and before substantial damage has been done to the ecosystem.

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* Citations are listed at the end of the Section. They can be located alphabetically within subtopics or by their superior numbers which run consecutively across subtopics for the entire Section.

Several potentially useful methods for rapid in-plant monitoring are being explored (Sparks et al. 1969,[7] Waller and Cairns 1969[8]), and one rapid in-stream method is now operational (Cairns et al. 1968,[2] Cairns and Dickson 1971[3]). These in-plant methods use changes in heart rate, respiration, and movements of fish within a container to detect sublethal concentrations of toxicants in a waste discharge. Continual information on toxicity of a waste should enable sanitary engineers to identify those periods likely to produce the most toxic wastes and to identify those components of the production process that contribute significantly to toxicity. This could be accomplished with bioassays as they are currently used, but rarely are enough samples taken over a period of time sufficient to give the range of information that would be available with continually operating bioassay techniques.

BIOASSAYS

Of equal importance to the river surveys and the in-plant and in-stream monitoring systems is the availability of toxicity information based on a predictive bioassay. The bioassay provides valuable information pertaining to the effects of potential or contemplated discharges on aquatic life. Acute bioassays are useful as a shortcut or predictive method of estimating safe concentrations by use of suitable application factors for many pollutants, as recommended throughout this Report.

However, determining only the acute lethal toxicity of wastes is no longer adequate. Good health and an ability to function vigorously are as important for aquatic ecosystems as they are for humans. The former end point of bioassays, viz., death, has been supplanted by more subtle end points such as the protection of respiration, growth, reproductive success, and a variety of other functional changes (Cairns 1967).[1] Acute toxicity determinations are being supplemented by long-term tests often involving an entire life cycle. The latter require more time and expense than short-term tests, but they provide better predictive information about biologically safe concentrations of various toxicants. Bioassays of organisms other than fish are becoming increasingly common because of the realization that elimination of the lower organisms can also have serious consequences.

SIMULATION TECHNIQUES

The fourth component now available to provide ecological information is the use of scale models. Models are used to study major ecological or environmental problems by simulating prospective new uses. Engineering scale models are common, but ecological scale models or environmental simulation systems are not yet as commonly used. Experimental streams and reservoirs have been constructed to predict toxicity of waste discharges, determine factors responsible for productivity of aquatic communities, and answer questions about plant site location (Haydu 1968,[6] Warren and Davis 1971[9]).

BIOASSAYS

Bioassays are used to evaluate a given pollutant in terms of existing water quality. Most pollution problems involve discharges of unknown and variable composition where more than one toxicant or stress is present. In evaluating criteria for specific toxicants, consideration must be given to other environmental influences such as dissolved oxygen, temperature, and pH.

Harmful effects of pollutants can be described by one or more of the following terms:

acute—involves a stimulus severe enough to bring about a response speedily, usually within four days for fish.
subacute—involves a stimulus less severe than an acute stimulus, producing a response in a longer time; may become chronic.
chronic—involves a lingering or continuous stimulus; often signifying periods of about one-tenth of the life span or more.
lethal—causes death by direct action.
sublethal—insufficient to cause death.
cumulative—brought about, or increased in strength, by successive additions.

Two broad categories of effect (Alderdice 1967)[10] may be distinguished: acute toxicity which is usually lethal, and chronic toxicity which may be lethal or sublethal.

MEASURES OF TOXICITY

Most of the available toxicity data are reported as the median tolerance limit (TLm or TL50) or median lethal concentration (LC50). Either symbol signifies the concentration that kills 50 per cent of the test organisms within a specified time span, usually in 96 hours. The customary 96-hour (four-day) time period is recommended as adequate for most routine tests of acute toxicity with fish. A threshold of acute toxicity will have been attained within this time in the majority of cases (Sprague 1969).[43] This *lethal threshold concentration* is usually noticeable in the data. Sometimes mortality continues, and tests of a week or longer would be necessary to determine the threshold. The lethal threshold concentration should be reported if it is demonstrated, because it is better for comparative purposes than the arbitrary 96-hour LC50. Absence of any apparent threshold is equally noteworthy.

The median lethal concentration is a convenient reference point for expressing the acute lethal toxicity of a given toxicant to the average or typical test animal. Obviously it is in no way a safe concentration, although occasionally the two have been confused. Safe levels, which permit reproduction, growth, and all other normal life-processes in the fish's natural habitat, usually are much lower than the LC50. In this book, the recommended criteria are intended to be safe levels.

Substantial data on long-term effects and safe levels are available for only a few toxicants. Information is now accumulating on the effect of toxicants on reproduction, an important aspect of all long-term toxicity tests. Other information is being gathered on sublethal effects on growth, performance, avoidance reactions, and social behavior of fish. Also important is the sensitivity of organisms at various life stages. Many organisms are most sensitive in the larval, nymphal, molting, or fry stage; some are most sensitive in the egg and sperm stage.

It would be desirable if a single, universal, rapid, biological test could be used to measure directly sublethal effects of a pollutant. Data on sublethal responses of fish have been used, such as respiratory rates and "coughing," swimming speed, avoidance behavior, and specific physiological and biochemical changes in various organisms; and histological studies have been made. A review of these (Sprague 1971)[46] shows that no single test is meaningful for all kinds of pollutants. Therefore, it is recommended that routine assessment and prediction of safe levels be made by carrying out bioassays for acute lethal toxicity and multiplying the lethal concentration by a suitable application factor. The application factors used and recommended here have been derived principally from chronic or sublethal laboratory experiments or from well documented field studies of polluted situations.

Acceptable concentrations of toxicants to which organisms are exposed continually must be lower than the higher concentrations that may be reached occasionally but briefly without causing damage. Both maximum short-time concentrations and the more restrictive range of safe concentrations for continuous exposure are useful. The recommendations in this Report are those considered safe for continuous exposure, although in some cases there has also been an indication of permissible higher levels for short periods.

In field situations and industrial operations, average 24-hour concentrations can be determined by obtaining composite or continuous samples. After 24 hours, the sample may be mixed and analyzed. The concentration found will represent the average concentration. Samples obtained this way are more reproducible and easier to secure than the instantaneous sample of maximum concentrations. However, average concentrations are of little significance if fish are killed by a sharp peak of concentration, and for that reason maximum concentrations must also be considered.

METHODS FOR BIOASSAYS

Although there are many types of assays, two are in general use:

1. the static bioassay in which the organisms are held in a tank containing the test solution, and
2. the continuous flow or flow-through bioassay in which the test solution is renewed continually.

The difference between the two types is not always great, but one can have clear advantages over the other.

An outline of methods for routine bioassays has been given in "Standard Methods for the Examination of Water and Wastewater" (American Public Health Association, American Water Works Association, Water Pollution Control Federation, 1971,[11] hereafter referred to as Standard Methods 1971[49]). Cope (1961)[21] described bioassay reporting, and Cairns (1969)[20] presented a rating system for evaluating the quality of the tests. Sprague (1969,[43] 1970,[44] 1971[45]) reviewed research to develop more incisive testing methods. Their findings are utilized in this Report.

Procedure for acute bioassay with fish is now relatively standardized and usually incorporates:

- a series of replicate test containers, each with a different but constant concentration of the toxicant;
- a group of similar fish, usually 10, in each container;
- observations of fish mortality during exposures that last between one day and one week, usually four days; and
- final results expressed as LC50.

Other factors that are required for good bioassay practice are briefly summarized in the references mentioned above.

CHECKLIST FOR PROCEDURES

Species

A selected strain of fish or other aquatic organisms of local importance should be used in bioassays conducted for the purpose of pollution monitoring. Preferably it should be a game or pan fish, which are usually among the more sensitive. Ability to duplicate experiments is enhanced by the use of a selected strain of test organisms (Lennon 1967).[31] A selected strain can also help to determine the difference between toxicants more reliably, and to detect discrepancies in results due to apparatus. A National Research Council subcommittee chaired by Dr. S. F. Snieszko is currently preparing a report, *Standards and guidelines for the breeding, care, and management of laboratory animals—Fish*, which will be useful in this area. Susceptibility to toxicants among different species of fish is generally less than might be expected—sometimes no greater than when a single species is tested in different types of water. For example, trout and certain coarse fishes were equally resistant to ammonia when tests continued for several days to give the less sensitive species time to react (Ball 1967a)[13] and even for zinc, the coarse fishes were no more than 3.5 times as resistant as trout (Ball 1967b).[14] Recommendations for the selected test fish will often provide protection to other aquatic animals and plants. There are exceptions to this generalization: for example, copper is quite damaging to algae and mollusks, and insecticides are especially dangerous to aquatic arthropods. Sufficient data exist to predict these situations. When they are expected, bioassays should be run with two kinds of invertebrates and two kinds of algae (Patrick et al. 1968).[41]

In the case of important bodies of water, there is good reason to test several kinds of aquatic organisms in addition to fish. Patrick et al. (1968)[41] made a comparative study of the effects of 20 pollutants on fish, snails, and diatoms and found that no single kind of organism was most sensitive in all situations. The short-term bioassay method for fish may also be used for many of the larger invertebrate animals. A greater volume of test water and rate of flow, or both, may be required in relation to weight of the animals since their metabolic rate is higher on a weight basis.

Larvae of mollusks or crustaceans can be good test animals. The crustacean *Daphnia* is a good test animal and was widely used in comparative studies of toxicants by Anderson (1950).[12] Recently Biesinger and Christensen (unpublished data, 1971)[15] have carried out tests on the chronic effects of toxicants on growth, survival, and reproduction of *Daphnia magna*. Because of the rapid life cycle of *Daphnia*, experiments on chronic toxicity can be completed in about the same time as an acute toxicity test with fish.

Patrick et al. (1968)[41] have shown that diatoms, snails and fish exposed for roughly comparable periods of time and in similar environmental conditions very often have similar LC50's, but at other times these may differ greatly.

However, for some toxicants diatoms were most sensitive; for others, fish; and for others, snails. When one is comparing data of this type, one questions whether a LC50 for a diatom population in which a number of divisions have occurred during the test period is comparable to that obtained for fish and snails in which no reproduction has occurred during the test period. In the sense that there are 50 per cent fewer cells in the LC50 concentration than there are in the diatom control culture, the test is somewhat equivalent to a test of acute toxicity that results in 50 per cent fewer surviving fish in the LC50 than in the control container. Also loss of ability to grow and divide might be just as fatal to a microbial population as death of a substantial number of its members would be to a fish population.

When the absolute time for the test is considered, there are also reasons for believing that exposure of diatoms to a toxicant through several generations might not constitute a chronic test, because it is quite possible that for toxicants to accumulate in a cell may require a period of exposure much more lengthy than that encompassed in the average test which only spans a few generations. This would be particularly true when the organisms were dividing rapidly and the additional protoplasm diluted the material being accumulated.

Dilution Water

Toxicants should be tested in the water that will receive the pollutant in question. In this way all modifying factors and combined toxicities will be present. It is not advisable to use tap water for dilution, because it may contain chlorine and other harmful materials such as copper, zinc, or lead from plumbing systems. Routine dechlorination does not insure complete removal of chlorine.

Variations in physical and chemical characteristics of water affect toxicity of pollutants. Effects of five environmental entities on the lethal threshold of ammonia were illustrated a decade ago (Lloyd 1961b).[31] Hardness of water is particularly important in toxicity of metals. Hydrogen ion concentration is an important modifying factor for ammonia and cyanide. Higher temperatures sometimes increase toxicity of a pollutant, but recent work shows that phenol, hydrogen cyanide, ammonia, and zinc may be more toxic at low temperatures (United Kingdom Ministry of Technology 1969).[50] Dissolved oxygen levels that are below saturation will increase toxicity, and this is predictable (Lloyd 1961a;[30] Brown 1968).[16]

The supply of dilution water must be adequate to maintain constant test conditions. In both static and continuous flow tests, a sufficiently large volume of test water must be used, and it must be replaced or replenished frequently. This is to provide oxygen for the organism and dilution of metabolic wastes, to limit changes in temperature and pH, and to compensate for degradation, volatilization, intake, and sorption of the toxicant. In static tests, there should be two or three liters of water per gram of fish, changed daily, or increased proportionally in volume for the number of days of the test. In continuous flow tests, the flow must provide at least two or three liters of water per gram of fish per day, and it must equal test-volume in five hours or less, giving 90 per cent replacement in half a day or less.

Acclimation

Acclimatizing the test organism to the specific water before the bioassay begins may have marked effect upon the outcome. Abrupt changes in quality of the water should be avoided. Time for acclimation of the organisms to the conditions of the diluent water should be as generous as possible, dependent on life span. At least two weeks is recommended for fish.

Test Methods

Test methods must be adequately described when the results are given. Several bioassay procedures are listed in Table III-1. Adequate and appropriate control tests must always be run (Sprague 1969).[43] Survival of the control organisms is a minimum indication of the quality of the test organisms. In addition, levels of survival and health in holding tanks should be indicated and the conclusions recorded.

TABLE III-1—Recommended Literature Sources for Bioassay and Biomonitoring Procedures with Various Aquatic Organisms

Kind of organism	Type of response	Appropriate situations for use	Reference
Fish and Macroinvertebrates	96-hour lethal concentration	To measure lethal toxicity of a waste of known or unknown composition. To serve as a foundation for extrapolating to presumably safe concentrations. To monitor industrial effluents.	Standard Methods 1971[46]
Fish and macroinvertebrates	Lethal threshold concentration	For research applications to document lethal thresholds.	Sprague 1969,[43] 1970[44]
Fish and invertebrates	Incipient lethal temperatures & ultimate incipient lethal temperatures	For research to determine lethal temperature ranges of a given species.	Fry 1947,[20] Brett 1952[13]
Fish	Respiratory movements as acute sublethal response	Quick (1-day) indication of possible sublethal effects. For research and monitoring.	Schaumburg et al. 1967[40]
Fish (i.e., fathead minnows, brook trout, bluegill)	Reproduction, growth, and survival	Chronic tests for research on safe concentrations.	Mount 1968,[37] Mount & Stephan 1967,[38] Brungs 1969,[15] McKim & Benoit 1971,[34] Eaton 1970[19]
Daphnia	Survival, growth, and reproduction	Rapid completion of chronic tests for testing special susceptibility of crustaceans	Anderson 1956,[11] Biesinger & Christensen (Unpublished data)[12]
Diatoms	Survival, growth, and reproduction	A sensitive, rapid, chronic test for research, prediction, or monitoring*	Patrick 1968[39]
Marine crustaceans, larvae mollusks	Survival, growth, and development through immature stages	A sensitive, rapid, chronic test for research, prediction, or monitoring*	Woelke 1967[51]

* requires an operator with some specialized biological training.

Dissolved Oxygen

The problem of maintaining dissolved oxygen concentrations suitable for aquatic life in the test water can be difficult. The suggestions on test volume and replacement times (see Dilution Water above) should provide for adequate oxygen in most cases. However, with some pollutants, insufficient oxygen may be present in the test water because a biochemical and a chemical oxygen demand (BOD and COD) may consume much of the available dissolved oxygen. Aeration or oxygenation may degrade or remove the test material. Devices for maintaining satisfactory dissolved oxygen in static tests have been proposed and used with some degree of effectiveness, and are described in Doudoroff et al. (1951).[22]

Concentrations

Periodic measurements of concentration of the toxicant should be made at least at the beginning and end of the bioassay. If this is not possible, introduced concentrations may be stated alone, but it should be realized that actual concentrations in the water may become reduced.

In the flow-through type of bioassay, a large quantity of test water can be made up and used gradually. More often a device is used to add toxicant to a flow of water, and the mixture is discharged into the test container, using apparatus such as "dipping bird" dosers described by Brungs and Mount (1967).[19] Other devices have been developed by Stark (1967),[47] and Mount and Warner (1965),[39] using the doser technique.

Evaluation of Results

Mortality rates at the longest exposure time should be plotted on a vertical probit scale against concentrations of toxicants on a horizontal logarithmic scale. The concentration which causes 50 per cent mortality can be read and used as LC50. Errors in LC50 can be estimated using the simple nomograph procedures described by Litchfield and Wilcoxon (1949).[33] A more refined estimate of error may be made using the methods of Finney (1952),[24] which can be programmed for a computer.

The value of the results would be improved if the LC50's were estimated (by the above procedures) at frequent exposure times such as 1, 2, 4, 8±1, 14±2, 24, 48, 72, and 96 hours. A toxicity curve of time versus LC50 could then be constructed on logarithmic axes. The lethal threshold concentration could then be estimated in many cases (Sprague 1969)[48] to provide a more valid single number for description of acute toxicity than the arbitrary 96-hour LC50.

For some purposes, such as basic research or situations where short exposures are of particular concern, it would be desirable to follow and plot separately the mortality of the group of fish in each tank. In this way, the median lethal time can be estimated for a given concentration. Methods for doing this are given in Appendix II–A.

APPLICATION FACTORS

Short-term or acute toxicity tests do not indicate concentrations of a potential toxicant that are harmless under conditions of long-term exposure. Nevertheless, for each toxicant there is obviously a numerical value for the ratio of the safe concentration to the acutely lethal concentration. Such values are called application factors. In some cases this safe-to-lethal ratio is known with reasonable accuracy from experimental work, as in the examples given in Table III–2. However, for most toxicants, the safe level has not been determined, and must be predicted by some approximate method. In these cases, the assumption has been made in this Report, that the numerical value of the safe-to-lethal ratio, the application factor, is constant for related groups of chemicals. Values for the ratio will be recommended. The safe level of a particular toxicant can then be estimated approximately by carrying out an acute bioassay to determine the lethal concentration, then multiplying this by the suggested application factor. An application factor does not make allowance for unknown factors. It is merely a fractional or decimal factor applied to a lethal concentration to estimate the safe concentration.

Ideally, an application factor should be determined for each waste material in question. To do this, it is necessary first to determine the lethal concentration of the waste according to the bioassay procedures outlined above. To obtain the application factor, the safe concentration of the same waste must be determined for the same species by thorough research on physiological, biochemical, and behavioral effects, and by studying growth, reproduction, and production in the laboratory and field. The safe-to-lethal ratio obtained could then be used as an application factor in a given situation, by working from the measured LC50 of a particular kind of waste to predict the safe concentration.

TABLE III–2—Ratios between the safe concentration and the lethal concentration which have been determined experimentally for potential aquatic pollutants. Sources of data are given in the sections on the individual pollutants.

Material	Species of animal	Safe-to-lethal ratio
LAS	Fathead minnow (Pimephales promelas)	Between 0.14 and 0.28 (average 0.21)
Chlorine	Fathead minnow	0.16
	Gammarus	0.16
Sulfides	Fathead minnow and white sucker (Catostomus commersoni)	0.1±
	Walleye pike (Stizostedion vitreum v.)	0.22±
Copper	Several species of fish	[illegible] to 0.1
Tr[illegible] chromium	Fathead minnow	0.037
Hexavalent chromium	Fathead minnow	0.03
	Brook trout (Salvelinus fontinalis)	0.012
	Rainbow trout (Salmo gairdneri)	0.04
Malathion	Fathead minnow and bluegill (Lepomis macrochirus)	0.03
Carbaryl	Fish species	0.02
Nickel	Fathead minnow	0.02
Lead	Rainbow and Brook trout	<0.02
Zinc	Fathead minnow	0.005

In this approach, a 96-hour LC50 is determined for the pollutant using water from the receiving stream for dilution. The test organisms selected should be among the most sensitive species, or an important local species at a sensitive life stage, or a species whose relative sensitivity is known. This procedure takes into consideration the effects of local water quality and the stress or adverse effects of wastes already present in the stream. The LC50 thus found is then multiplied by the application factor for that waste to determine its safe concentration in the specific stream or section of stream. Such bioassays should be repeated at least monthly or when changes in process or rate of waste discharge are observed.

For example, if the 96-hour LC50 is 0.5 milligrams per liter (mg/l) and the concentration of the waste found to be safe is 0.01 mg/l, the ratio would be:

$$\frac{\text{Safe Concentration}}{\text{96-hour LC50}} = \frac{0.01}{0.50} = \frac{1}{50}$$

In this instance, the safe-to-lethal ratio is 0.02. It can be used as an application factor in other situations. Then, in a given situation involving this waste, the safe concentration in the receiving stream would be found by multiplying the four-day LC50 by 0.02.

This predictive procedure based on lethal concentrations is useful, because the precise safe level of many pollutants is not known because of the uncertainty about toxicity of mixed effluents and the difference in sensitivity among fish and fish food organisms. Henderson (1957)[27] and Tarzwell (1962)[49] have discussed various factors involved in developing application factors. Studies by Mount and Stephan (1967),[38] Brungs (1969),[18] Mount (1968),[37] McKim and Benoit (1971),[36] and Eaton (1970)[24] in which continuous exposure was used, reveal that the safe-to-lethal ratio that permits spawning ranges over nearly two orders of magnitude. Exposure will not be constant in most cases, and higher concentrations usually can be tolerated for short periods.

Lethal threshold concentrations, which may require more than 96-hour exposures, may be beneficially used (Sprague 1969)[43] to replace 96-hour LC50 in the above procedures, and there is a trend today to use such threshold concentrations (Eaton 1970).[24]

At present, safe levels have been determined for only a few wastes, and as a result only a few application factors are known. Because the determination of safe levels of pollutants is an involved process, interim procedures for estimating tolerable concentrations of various wastes in receiving waters must be used. To meet this situation, three universal application factors selected on the basis of present knowledge, experience, and judgment are recommended at the end of this section. Where toxicants have a nonpersistent nature (a half life of less than 4 days) or noncumulative effects, an application factor of 0.1 of the 96-hour LC50 should not be exceeded at any time or place after mixing with the receiving waters. The 24-hour average of the concentration of these toxicants should not exceed 0.05 of the LC50 if aquatic life is to be protected. For toxic materials which are persistent or cumulative the concentrations should not exceed 0.05 of the 96-hour LC50 at any time or place, and the 24-hour average concentration should not exceed 0.01 of the 96-hour LC50 in order to protect aquatic life. It is proposed that these general application factors be applied to LC50 values determined in the manner described above to set tolerable concentrations of wastes in the receiving stream.

MIXTURES OF TWO OR MORE TOXICANTS

The toxicity of a mixture of pollutants may be estimated by expressing the actual concentration of each toxicant as a proportion of its lethal threshold concentration (usually equal to the 96-hour LC50) and adding the resulting numbers for all the toxicants. If the total is 1.0 or greater, the mixture will be lethal.

The system of adding different toxicants in this way is based on the premise that their lethal actions are additive. Unlikely as it seems, this simple rule has been found to govern the combined lethal action of many pairs and mixtures of quite dissimilar toxicants, such as copper and ammonia, and zinc and phenol in the laboratory (Herbert and Vandyke 1964,[29] Jordan and Lloyd 1964,[30] Brown et al. 1969).[17] The rule holds true in field studies (Herbert 1965,[28] Sprague et al. 1965).[45] The method of addition is useful and reasonably accurate for predicting thresholds of lethal effects in mixtures.

There is also evidence of a lower limit for additive lethal effects. For ammonia and certain other pollutants, levels below 0.1 of the lethal concentration do not seem to contribute to the lethal action of a mixture (Brown et al. 1969,[17] Lloyd and Orr 1969).[35] This lower cutoff point of 0.1 of the LC50 should be used when it is necessary to assess the lethal effects of a mixture of toxicants.

SUBLETHAL EFFECTS

Sublethal or chronic effects of mixtures are of great importance. Sublethal concentrations of different toxicants should be additive in effect. Here again, it would be expected that for any given toxicant there would be some low concentration that would have no deleterious effect on an organism and would not contribute any sublethal toxicity to a mixture, but there is little research on this subject. Biesinger and Christensen (*unpublished data* 1971),[52] concluded that subchronic concentrations of 21 toxicants were close to being additive in causing chronic effects on reproduction in *Daphnia*. Copper and zinc concentrations of about 0.01 of the LC50 are additive in causing avoidance reactions (Sprague et al. 1965).[45] On the other hand, somewhat lower metal concentrations of about 0.003 of the LC50 do not seem to be additive in affecting reproduction of fish

(Eaton *unpublished data* 1971).[33] Perhaps there is a lower cutoff point than 0.01 of the LC50 for single pollutants contributing to sublethal toxicity of a mixture.

As an interim solution, it is recommended that the contribution of a single pollutant to the sublethal toxicity of a mixture should not be counted if it is less than 0.2 of the recommended level for that pollutant. Applying this to a basic recommended level of 0.05 (see the Recommendation that follows) of the LC50 would yield a value of 0.01 of the LC50, corresponding to the possible cutoff point suggested above.

It is expected that certain cases of joint toxicity will not be covered by simple addition. The most obvious exception would be when two toxicants combine chemically. For example, mixed solutions of cyanides and metals could cause addition of toxicity or very different effects if the metal and cyanide combined (Doudoroff et al. 1966).[23] A thorough understanding of chemical reactions is necessary in these cases.

For further discussions of bioassays and the difficulties posed in assessing sublethal effects of toxicants on organisms, see Section IV, pp. 233–237.

Recommendations for the Use of Application Factors to Estimate Safe Concentrations of Toxic Wastes in Receiving Streams

Where specific application factors have been determined for a given material, they should be used instead of the safe concentration levels of wastes given below:

(a) Concentration of materials that are nonpersistent or have noncumulative effects should not exceed 0.1 of the 96-hour LC50 at any time or place after mixing with the receiving waters. The 24-hour average of the concentration of these materials should not exceed 0.05 of the LC50 after mixing.

(b) For toxicants which are persistent or cumulative, the concentrations should not exceed 0.05 of the 96-hour LC50 at any time or place, nor should the 24-hour average concentration exceed 0.01 of the 96-hour LC50.

(c) When two or more toxic materials are present at the same time in the receiving water, it should be assumed unless proven otherwise that their individual toxicities are additive and that some reduction in the permissible concentrations is necessary. The amount of reduction required is a function of both the number of toxic materials present and their concentrations in respect to the permissible concentrations. The following relationship will assure that the combined amounts of the several substances do not exceed a permissible concentration:

$$\frac{C_a}{L_a}+\frac{C_b}{L_b}+\cdots+\frac{C_n}{L_n}\leq 1.0$$

This formula may be applied where C_a, C_b, . . . C_n are the measured or expected concentrations of the several toxic materials in the water, and L_a, L_b, . . . L_n are the respective concentrations recommended or those derived by using recommended application factors on bioassays done under local conditions. Should the sum of the several fractions exceed 1.0. a local restriction on the concentration of one or more of the substances is necessary.

C and L can be measured in any convenient chemical unit as proportions of the LC50 or in any other desired way, as long as the numerator and denominator of any single fraction are in the same units. To remove natural trace concentrations and low nonadditive concentrations from the above formula, any single fraction which has a value less than 0.2 should be removed from the calculation.

Example:

Small quantities of five toxicants are measured in a stream as follows:

3 micrograms/liter (μg/l) of zinc; 3 μg/l of phenol; 3 μg/l of un-ionized ammonia as calculated from Figure III-10 (see Ammonia, p. 186); 1 μg/l of cyanide; and 1 μg/l of chlorine.

A bioassay with zinc sulphate indicates that the 96-hour LC50 is 1.2 mg/l. The application factor for zinc is 0.005; therefore, the allowable limit is 0.005× 1.2=0.006 mg/l. Initial bioassays with phenol, ammonia, and cyanide indicate that the recommended values are the safe concentrations stated in other sections of the Report, not the fractions of LC50; so the limits are 0.1 mg/l, 0.02 mg/l, and 0.005 mg/l. The permissible limit for chlorine (page 189) is 0.003 mg/l. Therefore, the total toxicity is estimated as follows for zinc, phenol, ammonia, cyanide, and chlorine, respectively:

$$\frac{0.003}{0.006}+\frac{0.003}{0.1}+\frac{0.003}{0.02}+\frac{0.001}{0.005}+\frac{0.001}{0.003}$$

$$=0.5+0.03+0.15+0.2+0.33$$

The second and third terms, i.e., phenol and ammonia, should be deleted since they are below the minimum of 0.2 for additive effects. This leaves $0.5+0.2+0.33=1.03$, indicating that the total sublethal effect of these three toxicants is slightly above the permissible level and that no higher concentration of any of the three is safe. Thus none can be added as a pollutant.

PHYSICAL MANIPULATION OF THE ENVIRONMENT

Numerous activities initiated to maximize certain uses of water resources often adversely affect water quality and minimize other uses. These activities have caused both benefit and harm in terms of environmental quality. The common forms of physical alteration of watersheds are channelization, dredging, filling, shoreline modifications (of lakes and streams), clearing of vegetation, rip-rapping, diking, leveling, sand and gravel removal, and impounding of streams.

Channelization is widespread throughout the United States, and many studies have been conducted documenting its effects. Channelization usually increases stream gradient and flow rates. The quiet areas or backwaters are either eliminated or cut off from the main flow of the stream, the stream bed is made smooth, thus reducing the habitats available to benthic organisms, and surrounding marshes and swamps are more rapidly drained. The steeper gradient increases velocity allowing the stream to carry a greater suspended load and causing increased turbidity. The rate of organic waste transformation per mile is usually reduced, and destruction of spawning and nursery areas often occurs. Trautman (1939),[67] Smith and Larimore (1963),[65] Peters and Alvord (1964),[64] Welker (1967),[68] Martin (1969),[63] and Gebhards (1970)[58] have discussed the harmful effects of channelization on some fish populations and the effect on stimulation of less desirable species.

Dredging undertaken to increase water depth often destroys highly productive habitats such as marshes (Marshall 1968,[62] Copeland and Dickens 1969).[56] The spoils from dredging activities are frequently disposed of in other shallow sites causing further loss of productive areas. For example, Taylor and Saloman (1968)[66] reported that since 1950 there has been a 20 per cent decrease in surface area of productive Boca Ciega Bay, Florida, due to fill areas. It has become common practice to fill in marshy sites near large metropolitan areas (e.g., San Francisco Bay, Jamaica Bay) to provide for airport construction and industrial development.

In addition to the material that is actually removed by the dredging process, a considerable amount of waste is suspended in the water resulting in high turbidities (Mackin 1961).[61] If the dredged sediments are relatively nontoxic, gross effects on motile aquatic life may not be noticeable, but benthic communities may be drastically affected by the increased redeposition of silt (Ingle 1952).[59]

In many instances either high nutrient or toxic sediments are suspended or deposited during the dredging process. This action may kill aquatic organisms by exposure to the toxicants present or by the depletion of dissolved oxygen concentrations, or both. Brown and Clark (1968)[54] noted a dissolved oxygen reduction of 16 to 83 per cent when oxidizable sediments were resuspended. In many cases disturbed sediments containing high nutrient concentrations may stimulate undesirable forms of phytoplankton or *Cladophora*. Gannon and Beeton (1969)[57] categorized harbor sediments in five groups. Those most severely polluted were toxic to various animals and did not stimulate growth of phytoplankton. Other sediments were toxic but stimulated plant growth. The least polluted sediments were not toxic and stimulated growth of phytoplankton but not *Cladophora*.

Three basic aspects must be considered in evaluating the impact of dredging and disposal on the aquatic environment: (1) the amount and nature of the dredgings, (2) the nature and quality of the environments of removal and disposal, and (3) the ecological responses. All vary widely in different environments, and it is not possible to identify an optimal dredging and disposal system. Consequently, the most suitable program must be developed for each situation. Even in situations where soil is deposited in diked enclosures or used for fill, care must be taken to monitor overflow, seepage, and runoff waters for toxic and stimulatory materials.

Artificial impoundments may have serious environmental impact on natural aquatic ecosystems. Dams and other artificial barriers frequently block migration and may destroy large areas of specialized habitat. Aquatic organisms are frequently subjected to physical damage if they are allowed to pass through or over hydroelectric power units and other man-made objects when properly designed barriers are not provided. At large dams, especially those designed for hydroelectric power, water drawn from the pool behind the dam is frequently taken from great depths, resulting in the release to the receiving stream of waters low in dissolved oxygen and excessively cold. This can be a problem, particularly in areas where nonnative fish are stocked.

Cutting down forests, planting the land in crops, and partially covering the surface of a watershed by building roads, houses, and industries can have detrimental effects on water ways. Wark and Keller (1963)[68] showed that in the Potomac River Basin (Washington, D.C.) reducing the forest cover from 80 per cent to 20 per cent increased the annual sediment yield from 50 to 400 tons per square mile per year. The planting of land in crops increased the sediment yield from 70 to 300 tons per square mile per year, or a fourfold increase as the land crops increased from 10 per cent to 50 per cent. Likens et al. (1970)[60] showed that cutting down the forest in the Hubbard Brook area (Vermont) caused substantial changes in the streams. The sediment load increased fourfold over a period from May 1966 to May 1968. Furthermore, the particulate matter drained from the deforested watershed became increasingly inorganic in content, thus reducing the value of the sediment as a food source. The nutrient content of the water was also affected by cutting down the forests. The nitrate concentration increased from 0.9 mg/l prior to the cutting of vegetation to 53 mg/l two years later. Temperatures of streams in deforested areas were higher, particularly during the summer months, than those of streams bordered by forests (Brown and Krygier 1970).[55]

Prior to any physical alterations of a watershed, a thorough investigation should be conducted to determine the expected balance between benefits and adverse environmental effects.

SUSPENDED AND SETTLEABLE SOLIDS

Suspended and settleable solids include both inorganic and organic materials. Inorganic components include sand, silt, and clay originating from erosion, mining, agriculture, and areas of construction. Organic matter may be composed of a variety of materials added to the ecosystem from natural and man-made sources. These inorganic and organic sources are discussed in the Panel Report on Marine Aquatic Life and Wildlife (Section IV), and the effects of land-water relationships are described in the report on Recreation and Aesthetics (Section I).

SOIL AS A SOURCE OF MINERAL PARTICLES

Soil structure and drainage patterns, together with the intensity and temporal distribution of rainfall that directly affect the kind and amount of protective vegetative cover, determine the susceptibility of a soil to erosion. Where rain occurs more or less uniformly throughout the year, protective grasses, shrubs, or trees develop (Leopold, et al. 1964).[78] Where rainfall occurs intermittently, as in arid areas, growth of protective plants is limited thus allowing unchecked erosion of soils.

Wetting and drying cause swelling and shrinking of clay soils and leave the surface susceptible to entrainment in surface water flows. Suspended soil particle concentrations in rivers, therefore, are at their peak at the beginning of flood flows. Data on the concentration of suspended matter in most of the significant streams of the United States are presented in the U.S. Geological Survey Water Supply Papers.

Streams transport boulders, rocks, pebbles, and sand by intermittent rolling motions, or by intermittent suspension and deposition as particles are entrained and later settled on the bed. Fine particles are held in suspension for long periods, depending on the intensity of the turbulence. Fine silt particles, when dispersed in fresh waters, remain almost continuously suspended, and suspension of dispersed clay mineral particles may be maintained even by the thermally induced motions in water. These fine mineral particles are the soil materials of greatest significance to the turbidity values of a particular water.

The suspended and settleable solids and the bed of a water body must be considered as interrelated, interacting parts. For example, Langlois (1941)[77] reported that in Lake Erie the average of 40 parts per million (ppm) of suspended matter in the water was found to change quickly to more than 200 ppm with a strong wind. He further explained that this increase is attributed to sediments resuspended by wave action. These sediments enter from streams or from shoreline erosion.

Suspended clay mineral particles are weakly cohesive in fresh river waters having either unusually low dissolved salt concentrations or high concentrations of multivalent cations. Aggregations of fine particles form and settle on the bed to form soft fluffy deposits when such waters enter a lake or impoundment. However, clay mineral particles are dispersed or only weakly cohesive in most rivers.

EFFECTS OF SUSPENDED PARTICLES IN WATER

The composition and concentrations of suspended particles in surface waters are important because of their effects on light penetration, temperature, solubility products, and aquatic life (Cairns 1968).[79] The mechanical or abrasive action of particulate material is of importance to the higher aquatic organisms, such as mussels and fish. Gills may be clogged and their proper functions of respiration and excretion impaired. Blanketing of plants and sessile animals with sediment as well as the blanketing of important habitats, such as spawning sites, can cause drastic changes in aquatic ecosystems. If sedimentation, even of inert particles, covers substantial amounts of organic material, anaerobic conditions can occur and produce noxious gases and other objectionable characteristics, such as low dissolved oxygen and decreases in pH.

Absorption of sunlight by natural waters is strongly affected by the presence of suspended solids. The intensity of light (I) at any distance along a light ray (L) is, for a uniform suspension, expressed by the formula:

$$I=I_0{}^{-kcL},$$

where I_0 is the intensity just below the water surface ($L = 0$),

k is the extinction coefficient for the suspended solids, and c is the concentration of suspended solids. L can be related to the water depth by the zenith angle, i, the angle of refraction, r, and the index of refraction of water, 1.33, by Snell's rule:

$$\sin r = \frac{\sin i}{1.33}$$

The depth, D, is $L \cos r$. Refraction makes the light path more nearly vertical under water than the sun's rays, except when the sun's rays are themselves normal to the water surface.

The growth of fixed and suspended aquatic plants can be limited by the intensity of sunlight. An example of the decrease in the photic zone was calculated for San Francisco Bay (Krone 1963),[78] where k was 1.18×10^3 square centimeters per gram. For a typical suspended solids concentration of 50×10^{-6} grams per cubic centimeter, for an algae requiring 20 foot candles or more for its multiplication, and under incident sunlight of 13,000 foot candles the photic zone did not exceed 1.1 meters. A reduction in suspended solids concentration to 20×10^{-6} g/cm^3 increased the maximum depth of the photic zone to 2.8 meters.

Because suspended particles inhibit the penetration of sunlight, water temperatures are affected, and increasing turbidity results in increasing absorption near the water surface so that turbid waters warm more rapidly at the surface than do clearer waters. Warming and the accompanying decrease in density stabilize water and may inhibit vertical mixing. Lower oxygen transfer value from air to water results when surface waters are heated. This action combined with inhibited vertical mixing reduces the rate of oxygen transfer downward. Still or slowly moving water is most affected.

The rate of warming, dT/dt, at any distance from the surface along a light path, L, in water having uniform suspended material is

$$\frac{dT}{dt} = -\left[\frac{Ikc}{\rho C}\right]^{-kcL}$$

where ρ is the water density and C is the specific heat of the water. This equation shows that an increase in suspended sediment concentration increases the rate of warming near the surface and decreases exponentially with depth. The biological significance of this relationship is in the effect on time of formation, vertical distribution of thermal stratification, and stability of the upper strata. Increasing turbidity could change the stratification patterns of a lake and thus change the temperature distribution, oxygen regime, and composition of the biological communities.

ADSORPTION OF TOXIC MATERIALS

Suspended mineral particles have irregular, large surface areas, with electrostatic charges. As a consequence, clay minerals may sorb cations, anions, and organic compounds. Pesticides and heavy metals may be absorbed on suspended clay particles and strongly held with them. The sorption of chemicals by suspended matter is particularly important if it leads to a buildup of toxic and radioactive materials in a limited area with the possibility of sudden release of these toxicants. One such example has been reported by Benoit et al. (1967).[70] Gannon and Beeton (1969)[71] reported that sediments with the following characteristics dredged from various harbors on the Great Lakes were usually toxic to various organisms: COD 42,000 mg/l, volatile solids 4,000 mg/l, ammonia 0.075 mg/g, phosphate-P 0.65 mg/g.

The capacity of minerals to hold dissolved toxic materials is different for each material and type of clay mineral. An example illustrates the magnitudes of sorptive capacities: the cation exchange capacity (determined by the number of negatively charged sites on clay mineral surfaces) ranges from a few milliequivalents per hundred grams (me/100 g) of mineral for kaolinite clay to more than 100 me/100 g for montmorillonite clay. Typical estuarial sediments, which are mixtures of clay, silt, and sand minerals, have exchange capacities ranging from 15 to 60 me/100 g (Krone 1963).[78] The large amounts of such material that enter many estuaries and lakes from tributary streams provide continually renewed sorptive capacity that removes materials such as heavy metals, phosphorus, and radioactive ions. The average new sediment load flowing through the San Francisco Bay-Delta system, for example, has a total cation exchange capacity of a billion equivalents per year.

The sorptive capacity effectively creates the large assimilative capacity of muddy waters. A reduction in suspended mineral solids in surface waters can cause an increase in the concentrations of dissolved toxic materials contributed by existing waste discharges.

EFFECTS ON FISH AND INVERTEBRATES

The surface of particulate matter may act as a substratum for microbial species, although the particle itself may or may not contribute to their nutrition. When the presence of particulate matter enables the environment to support substantial increased populations of aquatic microorganisms, the dissolved oxygen concentration, pH, and other characteristics of the water are frequently altered.

There are several ways in which an excessive concentration of finely divided solid matter might be harmful to a fishery in a river or a lake (European Inland Fisheries Advisory Commission, EIFAC 1965).[73] These include:

- acting directly on fish swimming in water in which solids are suspended, either killing them or reducing their growth rate and resistance to disease;
- preventing the successful development of fish eggs and larvae;

- modifying natural movements and migrations of fish;
- reducing the food available to fish;
- affecting efficiency in catching the fish.

With respect to chemically inert suspended solids and to waters that are otherwise satisfactory for the maintenance of freshwater fisheries, EIFAC (1965)[73] reported:

- there is no evidence that concentrations of suspended solids less than 25 mg/l have any harmful effects on fisheries;
- it should usually be possible to maintain good or moderate fisheries in waters that normally contain 25 to 80 mg/l suspended solids; other factors being equal, however, the yield of fish from such waters might be somewhat lower than from those in the preceding category;
- waters normally containing from 80 to 400 mg/l suspended solids are unlikely to support good freshwater fisheries, although fisheries may sometimes be found at the lower concentrations within this range;
- only poor fisheries are likely to be found in waters that normally contain more than 400 mg/l suspended solids.

In addition, although several thousand parts per million suspended solids may not kill fish during several hours or days exposure, temporary high concentrations should be prevented in rivers where good fisheries are to be maintained. The spawning grounds of most fish should be kept as free as possible from finely divided solids.

While the low turbidities reported above reflected values that should protect the ecosystem, Wallen (1951)[80] reported that fish can tolerate higher concentrations. Behavioral reactions were not observed until concentrations of turbidity neared 20,000 mg/l, and in one species reactions did not appear until turbidities reached 100,000 mg/l. Most species tested endured exposures of more than 100,000 mg/l turbidity for a week or longer, but these same fishes finally died at turbidities of 175,000 to 225,000 mg/l. Lethal turbidities caused the death of fishes within 15 minutes to two hours exposure. Fishes that succumbed had opercular cavities and gill filaments clogged with silty clay particles from the water.

In a study of fish and macroinvertebrate populations over a four-year period in a stream receiving sediment from a crushed limestone quarry, Gammon (1970)[74] found that inputs that increased the suspended solids load less than 40 mg/l (normal suspended solids was 38 to 41 mg/l and volatile suspended solids 16 to 30 mg/l) resulted in a 25 per cent reduction in macroinvertebrate density in the stream below the quarry. A heavy silt input caused increases of more than 120 mg/l including some decomposition of sediment, and resulted in a 60 per cent reduction in density of macroinvertebrates. Population diversity indices were unaffected because most species responded to the same degree. The standing crop of fish decreased dramatically when heavy sediment occurred in the spring; but fish remained in pools during the summer when the input was heavy and vacated the pools only after deposits of sediment accumulated. After winter floods removed sediment deposits, fish returned to the pools and achieved levels of 50 per cent of the normal standing crop by early June.

Not all particulate matter affects organisms in the same way. For example, Smith, et al. 1965)[79] found that the lethal action of pulp-mill fiber on walleye fingerlings (*Stizostedion vitreum vitreum*) and fathead minnows (*Pimephales promelas*) was influenced by the type of fiber. In 96-hour bioassays, mortality of the minnows in 2,000 ppm suspensions was 78 per cent in conifer groundwood, 34 per cent in conifer kraft, and 4 per cent in aspen groundwood. High temperatures and reduced dissolved oxygen concentrations increased the lethal action of fiber.

Buck (1956)[71] studied the growth of fish in 39 farm ponds having a wide range of turbidities. The ponds were cleared of fish and then restocked with largemouth black bass (*Micropterus salmoides*), bluegill (*Lepomis macrochirus*), and redear sunfish (*Lepomis microlophus*). After two growing seasons the yields of fish were:

• clear ponds (less than 25 mg/l suspended solids)	161.5 lb/acre
• intermediate (25–100 mg/l suspended solids)	94.0 lb/acre
• muddy (more than 100 mg/l suspended solids)	29.3 lb/acre

The rate of reproduction was also reduced by turbidity, and the critical concentration for all three species appeared to be about 75–100 mg/l. In the same paper, Buck reported that largemouth black bass (*Micropterus salmoides*), crappies (*Pomoxis*), and channel catfish (*Ictalurus punctatus*) grew more slowly in a reservoir where the water had an average turbidity of 130 mg/l than in another reservoir where the water was always clear.

Floating materials, including large objects as well as very fine substances, can adversely affect the activities of aquatic life. Floating logs shut out sunlight and interfere particularly with surface feeding fish. Logs may also leach various types of organic acids due to the action of water. If they have been sprayed with pesticides or treated chemically, these substances may also leach into the water. As the logs float downstream their bark often disengages and falls to the bed of the stream, disturbing benthic habitats. Aquatic life is also affected by fine substances, such as sawdust, peelings, hair from tanneries, wood fibers, containers, scum, oil, garbage, and materials from untreated municipal and industrial wastes, tars and greases, and precipitated chemicals.

Recommendations

- **The combined effect of color and turbidity should not change the compensation point more than 10 per cent from its seasonally established norm, nor should such a change place more than 10 per cent of the biomass of photosynthetic organisms below the compensation point.**

- **Aquatic communities should be protected if the following maximum concentrations of suspended solids exist:**

High level of protection	**25 mg/l**
Moderate protection	**80 mg/l**
Low level of protection	**400 mg/l**
Very low level of protection	**over 400 mg/l**

COLOR

The true color of a specific water sample is the result of substances in solution; thus it can be measured only after suspended material has been removed. Color may be of organic or mineral origin and may be the result of natural processes as well as manufacturing operations. Organic sources include humic materials, peat, plankton, aquatic plants, and tannins. Inorganic substances are largely metallic, although iron and manganese, the most important substances, are usually not in solution. They affect color as particles. Heavy-metal complexes are frequent contributors to the color problem.

Many industries (such as pulp and paper, textile, refining, chemicals, dyes and explosives, and tanning) discharge materials that contribute to the color of water. Conventional biological waste treatment procedures are frequently ineffective in removing color. On the other hand, such treatment processes have caused an accentuation of the level of color during passage through the treatment plant. Physicochemical treatment processes are frequently preferable to biological treatment if color removal is critical (Eye and Aldous 1968,[51] King and Randall 1970[53]).

The tendency for an accentuation of color to occur as a result of complexing of a heavy metal with an organic substance may also lead to problems in surface waters. A relatively color-free discharge from a manufacturing operation, may, upon contact with iron in a stream, produce a highly colored water that would significantly affect aquatic life (Hem 1960,[52] Stumm and Morgan 1962[56]).

The standard platinum-cobalt method of measuring color is applicable to a wide variety of water samples (Standard Methods 1971).[55] However, industrial wastes frequently produce colors dissimilar to the standard platinum-cobalt color, making the comparison technique of limited value. The standard unit of color in water is that level produced by 1 mg/l of platinum as chloroplatinate ion (Standard Methods 1971).[55] Natural color in surface waters ranges from less than one color unit to more than 200 in highly colored bodies of water (Nordell 1961).[54]

That light intensity at which oxygen production in photosynthesis and oxygen consumption by respiration of the plants concerned are equal is known as the compensation point, and the depth at which the compensation point occurs is called the compensation depth. For a given body of water this depth varies with several conditions, including season, time of day, the extent of cloud cover, condition of the water, and the taxonomic composition of the flora involved. As commonly used, the compensation point refers to that intensity of light which is such that the plant's oxygen production during the day will be sufficient to balance the oxygen consumption during the whole 24-hour period (Welch 1952).[57]

Recommendation

The combined effect of color and turbidity should not change the compensation point more than 10 per cent from its seasonally established norm, nor should such a change place more than 10 per cent of the biomass of photosynthetic organisms below the compensation point.

DISSOLVED GASES

DISSOLVED OXYGEN

Oxygen requirements of aquatic life have been extensively studied. Comprehensive papers have been presented by Doudoroff and Shumway (1967),[89] Doudoroff and Warren (1965),[91] Ellis (1937),[93] and Fry (1960).[94] (Much of the research on temperature requirements also considers oxygen, and references cited in the discussion of Heat and Temperature, p. 151, are relevant here.) The most comprehensive review yet to appear has been written by Doudoroff and Shumway for the Food and Agriculture Organization (FAO) of the United Nations (1970).[97] This FAO report provides the most advanced summary of scientific research on oxygen needs of fish, and it has served as a basis for most of the recommendations presented in this discussion. In particular, it provided the criteria for citing different levels of protection for fish, for change from natural levels of oxygen concentration, and for the actual numerical values recommended. Much of the text below has been quoted verbatim or condensed from the FAO report. Its recommendations have been modified in only two ways: the insertion of a floor of 4 mg/l as a minimum, and the suggestion that natural minima be assumed to be equal to saturation levels if the occurrence of lower minima cannot be definitely established. Doudoroff and Shumway covered oxygen concentrations below the floor of 4 mg/l; however, the 4 mg/l floor has been adopted in this report for reasons explained below.

Levels of Protection

Most species of adult fish can survive at very low concentrations of dissolved oxygen. Even brook trout (*Salvelinus fontinalis*) have been acclimated in the laboratory to less than 2 mg/l of O_2. In natural waters, the minimum concentration that allows continued existence of a varied fish fauna, including valuable food and game species, is not high. This minimum is not above 4 mg/l and may be much lower.

However, in evaluating criteria, it is not important to know how long an animal can resist death by asphyxiation at low dissolved oxygen concentrations. Instead, data on the oxygen requirements for egg development, for newly hatched larvae, for normal growth and activity, and for completing all stages of the reproductive cycle are pertinent. Upon review of the available research, one fact becomes clear: *any* reduction of dissolved oxygen can reduce the efficiency of oxygen uptake by aquatic animals and hence reduce their ability to meet demands of their environment. There is evidently no concentration level or percentage of saturation to which the O_2 content of natural waters can be reduced without causing or risking some adverse effects on the reproduction, growth, and consequently, the production of fishes inhabiting those waters.

Accordingly, no single, arbitrary recommendation can be set for dissolved oxygen concentrations that will be favorable for all kinds of fish in all kinds of waters, or even one kind of fish in a single kind of water. Any reduction in oxygen may be harmful by affecting fish production and the potential yield of a fishery.

The selection of a level of protection (Table III-3 is a socioeconomic decision, not a biological one. Once the level of protection is selected, appropriate scientific recommendations may be derived from the criteria presented in this discussion.

Basis for Recommendations

The decision to base the recommendations on O_2 concentration minima, and not on average concentrations, arises from various considerations. Deleterious effects on fish seem to depend more on extremes than on averages. For example, the growth of young fish is slowed markedly if the oxygen concentration falls to 3 mg/l for part of the day, even if it rises as high as 18 mg/l at other times. It could be an inaccurate and possibly controversial task to carry out the sets of measurements required to decide whether a criterion based on averages was being met.

A daily fluctuation of O_2 is to be expected where there is appreciable photosynthetic activity of aquatic plants. In such cases, the minimum O_2 concentration will usually be found just before daybreak, and sampling should be done at that time. Sampling should also take into account the possible differences in depth or width of the water body. The guiding principle should be to sample the places where aquatic organisms actually live or the parts of the habitat where they should be able to live.

Before recommendations are proposed, it is necessary to evaluate criteria for the natural, seasonal O_2 minimum from which the recommendations can be derived. Natural levels are assumed to be the saturation levels, unless scientific data show that the natural levels were already low in the absence of man-made effects.

Certain waters in regions of low human populations can still be adequately studied in their natural or pristine condition. In these cases the minimum O_2 concentration at different seasons, temperatures, and stream discharge volumes can be determined by direct observation. Such observed conditions can also be useful in estimating seasonal minima in similar waters in similar geographical regions where natural levels can no longer be observed because of waste discharges or other man-made changes.

In many populated regions, some or all of the streams and lakes have been altered. Direct determination of natural minima may no longer be possible. In these cases the assumption of year-round saturation with O_2 is made in the absence of other evidence.

Supersaturation of water with dissolved oxygen may occur as the result of photosynthesis by aquatic vegetation. There is some evidence that this may be deleterious to aquatic animals because of gas bubble disease (see Total Dissolved Gases, p. 135).

Despite the statements in previous paragraphs that there is no single O_2 concentration which is *favorable* to all species and ecosystems, it is obvious that there are, nevertheless, very low O_2 concentrations that are *unfavorable* to almost all aquatic organisms. Therefore, a floor of 4 mg/l is recommended except in situations where the natural level of dissolved oxygen is less than 4 mg/l in which case no further depression is desirable. The value of 4 mg/l has been selected because there is evidence of subacute or chronic damage to several fish below this concentration. Doudoroff and Shumway (1970)[90] review the work of several authors as given below, illustrating such damage. Fathead minnows (*Pimephales promelas*) held at 4 mg/l spawned satisfactorily; only 25 per cent of the resultant fry survived for 30 days, compared to 66 per cent survival at 5 mg/l. At an oxygen level of 3 mg/l, survival of fry was even further reduced to 5 per cent (Brungs 1972[101] *personal communication*). Shumway et al. (1964)[98] found that the dry weight of coho salmon (*Oncorhynchus kisutch*) alevins (with yolk sac removed) was reduced by 59 per cent when they had been held at 3.8 mg/l of oxygen, compared to weights of the controls. The embryos of sturgeon (*Acipenser*) suffered complete mortality at oxygen concentrations of 3.0 to 3.5 mg/l, compared to only 18 per cent mortality at 5.0 to 5.5 mg/l (Yurovitskii 1964).[100] Largemouth bass (*Micropterus salmoides*) embryos reared at 25 C showed survival equal to controls only at oxygen levels above 3.5 mg/l (Dudley 1969).[92] Efficiency of food conversion by juvenile bass was nearly independent of O_2 at 5 mg/l and higher, but growth rate was reduced by 16.5 per cent at 4 mg/l, and 30 per cent at 3 mg/l (Stewart et al. 1967).[99] Similar reductions in growth of underyearling coho salmon occurred at the same O_2 concentrations (Herrmann et al. 1962).[95] Although many other experiments have shown little or no damage to performance of fish at 4 mg/l, or lower, the evidence given above shows appreciable effects on embryonic and juvenile survival and growth for several species of fish sufficient to justify this value.

Warm- and Coldwater Fishes

There are many associations and types of fish fauna throughout the country. Dissolved oxygen criteria for coldwater fishes and warmwater game fishes are considered together in this report. There is no evidence to suggest that the more sensitive warmwater species have lower O_2 requirements than the more sensitive coldwater fishes. The difference in O_2 requirements is probably not greater than the difference of the solubility of O_2 in water at the maximum temperatures to which these two kinds of fish are normally exposed in summer (Doudoroff and Shumway 1970).[90] In warmwater regions, however, the variety of fishes and fish habitats is relatively great, and there are many warmwater species that are exceedingly tolerant of O_2 deficiency.

Unusual Waters

There are certain types of waters that naturally have low oxygen content, such as the "black waters" draining swamps of the Southeastern United States. (Other examples include certain deep ocean waters and eutrophic waters that support heavy biomass, the respiration of which reduces O_2 content much of the time.) A special situation prevails in the deep layers (hypolimnion) of some lakes. Such layers do not mix with the surface layers for extended periods and may have reduced O_2, or almost none. Fish cannot live in the deep layers of many such lakes during a large part of the year, although each lake of this kind must be considered as a special case. However, the recommendation that *no* oxygen-consuming wastes should be released into the deep layers still applies, since there may be no opportunity for reaeration for an entire season.

Organisms Other Than Fish

Most research concerning oxygen requirements for freshwater organisms deals with fish; but since fish depend upon other aquatic species for food, it is necessary to consider the O_2 requirements of these organisms. This Section makes the assumption that the O_2 requirements of other components of the aquatic community are compatible with fish (Doudoroff and Shumway 1970).[90] There are certain exceptions where exceedingly important invertebrate organisms may be very sensitive to low O_2, more sensitive than the fish species in that habitat (Doudoroff and Shumway 1970).[90]

The situation is somewhat more complicated for invertebrates and aquatic plants, inasmuch as organic pollution that causes reduction of O_2 also directly increases food material. However, it appears equally true for sensitive invertebrates as for fish that *any* reduction of dissolved O_2 may have deleterious effects on their production. For example, Nebeker (1972)[97] has found that although a certain mayfly (*Ephemera simulans*) can survive at 4.0 mg/l of oxygen for four days, any reduction of oxygen below saturation causes a decrease in successful transformation of the immature to the adult stage.

Salmonid Spawning

For spawning of salmonid fishes during the season when eggs are in the gravel, there are even greater requirements for O_2 than those given by the high level of protection. (See Table III–3 for description of levels.) This is because the water associated with the gravel may contain less oxygen than the water in the stream above the gravel. There is abundant evidence that salmonid eggs are adversely affected in direct proportion to reduction in O_2. The oxygen criteria for eggs should be about half way between the nearly maximum and high levels of protection.

TABLE III–3—Guidelines for Selecting Desired Type and Level of Protection of Fish Against Deleterious Effects of Reduced Oxygen Concentrations

Level of protection	Intended type of protection	Possible application
Nearly Maximum[a]	For virtually unimpaired productivity and unchanged quality of a fishery.	Appropriate for conservation areas, parks, and water bodies of high or unique value. Requires, practically speaking, that little or no deoxygenating wastes be added to natural waters. Nor must there be any activities such as undesirable land use which would reduce the levels.
High	Not likely to cause appreciable change in the ecosystem, nor material reduction of fish production. Some impairment is risked, but appreciable damage is not to be expected at these levels of oxygen.	Could be appropriate for fisheries or aquatic ecosystems of some importance, which should not be impaired by other uses of water.
Moderate	Fisheries should persist, usually with no serious impairment, but with some decrease in production.	Could be used for fisheries which are valued, but must co-exist with major industries or dense human population.
Low	Should permit the persistence of sizeable populations of tolerant species and successful passage of most migrants[b]. Much reduced production or elimination of sensitive fish is likely.	Appropriate for fisheries that have some commercial or recreational value, but are so unimportant compared with other water uses, that their maintenance cannot be a major objective of pollution control. This type of protection should, however, provide for survival of sensitive species in adult or subadult life stages for short periods during the year, if oxygen levels at other times are satisfactory for growth, reproduction, etc.

[a] Note that there could be a higher level of protection that would require oxygen to be near natural level at all times, whereas nearly maximum requires only that oxygen should not fall below the lowest level characteristic of the season.

[b] But will not permit migrating salmonids, which would require at least a Moderate level of protection, for zones of passage.

Interaction with Toxic Pollutants or Other Environmental Factors

It is known that reduced oxygen levels increase the toxicity of pollutants. A method for predicting this interaction has been given by Brown (1968),[illegible] and a theoretical background by Lloyd (1961).[illegible] The disposal of toxic pollutants must be controlled so that their concentrations will not be unduly harmful at prescribed acceptable levels of O_2, temperature, and pH. The levels of oxygen recommended in this Section are independent of the presence of toxic wastes, no matter what the nature of the interaction between these toxicants and O_2 deficiencies. Carbon dioxide is an exception, because its concentration influences the safe level of oxygen. The recommendations for O_2 are valid when the CO_2 concentration is within the limits recommended in the section on CO_2.

Application of Recommendations

As previously stated, the recommendations herein differ in two important respects from those widely used. First, they are not fixed values independent of natural conditions. Second, they offer a choice of different levels of protection of fishes, the selection of any one of which is primarily a socioeconomic decision, not a biological one.

Table III–4 presents guidelines for the protection of fishes at each of four levels. Each column shows the level to which the dissolved O_2 can be reduced and still provide the stated level of protection for local fisheries. The values can be derived from the equations given in the recommendations. These equations have been calculated to fit the curves shown in the figure on page 264 of Doudoroff and Shumway (1970),[illegible] which serve as the basis of the recommendations. To use Table III–4, the estimated natural seasonal minimum should first be determined on the basis of available data or from expert judgment. This may be taken to be the minimum saturation value for the season, unless there is scientific evidence that losses of O_2 levels prevailed naturally. The word "season" here means a period based on local climatic and hydrologic conditions, during which the natural thermal and dissolved O_2 regime of a stream or lake can be expected to be fairly uniform. Division of the year into equal three-month periods such as December–February, March–May, is satisfactory. However, under special conditions, the designated seasons should be periods longer or shorter than three months, and should in fact be taken as individual months. The selected periods need not be equal in length.

When the lowest natural value for the season has been estimated, the desired kind and level of protection should then be selected according to the guidelines in Table III–3. The recommended minimum level of dissolved oxygen may then be found in the selected column of Table III–4, or as given by the formula in the recommendation.

TABLE III-4—Example of Recommended Minimum Concentrations of Dissolved Oxygen

Estimated natural seasonal minimum concentration of oxygen in water	Corresponding temperature of oxygen-saturated fresh water	Recommended minimum concentrations of O_2 for selected levels of protection			
		Nearly maximal	High	Moderate	Low
5	(a) (a)	5	4.7	4.2	4.0
6	45C(a) (113F)(a)	6	5.5	4.8	4.0
7	35C (95.0F)	7	6.4	5.3	4.0
8	27.5C (81.5F)	8	7.1	5.8	4.3
9	21C (69.8F)	9	7.7	6.2	4.5
10	16C (60.8F)	10	8.2	6.5	4.7
12	7.7C (45.9F)	12	9.0	6.8	4.8
14	1.5C (34.7F)	14	9.3	6.8	4.9

a Included to cover waters that are naturally somewhat deficient in O_2. A saturation value of 5 mg/l might be found in warm springs or very saline waters. A saturation value of 6 mg/l would apply to warm sea water (32 C—90 F).

Note: The desired kind and level of protection of a given body of water should first be selected (across head of table). The estimated seasonal minimum concentration of dissolved oxygen under natural conditions should then be determined on the basis of available data, and located in the left hand column of the table. The recommended minimum concentration of oxygen for the season is then taken from the table. All values are in milligrams of O_2 per liter. Values for natural seasonal minima other than those listed are given by the formula and qualifications in the section on recommendations.

Examples

- It is desired to give moderate protection to trout (*Salvelinus fontinalis*) in a small stream during the summer. The maximum summer temperature is 20 C (68 F); the salt content of the water is low and has negligible effect on the oxygen saturation value. The atmospheric pressure is 760 millimeters (mm) Hg. Oxygen saturation is therefore 9.2 mg/l. This is assumed to be the natural seasonal minimum in the absence of evidence of lower natural concentrations. Interpolating from Table III-4 or using the recommended formula, reveals a minimum permissible concentration of oxygen during the summer of 6.2 mg/l. If a high level of protection had been selected, the recommendation would have been 7.8 mg/l. A low level of protection, providing little or no protection for trout but some for more tolerant fish, would require a recommendation of 4.5 mg/l. Other recommendations would be calculated in a similar way for other seasons.
- It is decided to give moderate protection to largemouth bass (*Micropterus salmoides*) during the summer. Stream temperature reaches a maximum of 35 C (95 F) during summer, and lowest seasonal saturation value is accordingly 7.1 mg/l. The recommendation for minimum oxygen concentration is 5.4 mg/l.
- For low protection of fish in summer in the same stream described above (for largemouth bass), the recommendation would be 4.0 mg/l, which is also the floor value recommended.
- It is desired to protect marine fish in full-strength sea water (35 parts per thousand salinity) with a maximum seasonal temperature of 16 C (61 F). The saturation value of 8 mg/l is assumed to be the natural dissolved oxygen minimum for the season. For a high level of protection, the recommendation is 7.1 mg/l, for a moderate level of protection it is 5.8 mg/l, and for a low level of protection it is 4.3 mg/l.

It should be stressed that the recommendations are the minimum values for any time during the same season.

Recommendations

(a) For nearly maximal protection of fish and other aquatic life, the minimum dissolved oxygen in any season (defined previously) should not be less than the estimated natural seasonal minimum concentration (defined previously) characteristic of that body of water for the same season. In estimating natural minima, it is assumed that waters are saturated, unless there is evidence that they were lower in the absence of man-made influences.

(b) For a high level of protection of fish, the minimum dissolved oxygen concentration in any season should not be less than that given by the following formula in which M = the estimated natural seasonal minimum concentration characteristic of that body of water for the same season, as qualified in (a):

$$\text{Criterion}^{*} = 1.41M - 0.0476M^2 - 1.11$$

(c) For a moderate level of protection of fish, the minimum dissolved oxygen concentration in any season should not be less than is given by the following formula with qualifications as in (b):

$$\text{Criterion}^{*} = 1.08M - 0.0415M^2 - 0.202$$

(d) For a low level of protection of fish, the minimum O_2 in any season should not be less than given by the following formula with qualifications as in (b):

$$\text{Criterion}^{*} = 0.674M - 0.0264M^2 + 0.577$$

(e) A floor value of 4 mg/l is recommended except in those situations where the natural level of dissolved oxygen is less than 4 mg/l, in which case no further depression is desirable.

(f) For spawning grounds of salmonid fishes, higher O_2 levels are required as given in the following formula with qualifications as in (b):

$$\text{Criterion}^{*} = 1.19M - 0.0242M^2 - 0.418$$

(g) In stratified eutrophic and dystrophic lakes, the dissolved oxygen requirements may not apply to the hypolimnion and such lakes should be considered on a case by case basis. In other stratified lakes, recommendations (a), (b), (c), and (d) apply; and if the oxygen is below 4 mg/l, recommendation (e) applies. In unstratified lakes recommendations apply to the entire circulating water mass.

(i) All the foregoing recommendations apply to all waters except waters designated as mixing zones

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* All values are instantaneous, and final value should be expressed to two significant figures.

(see section on Mixing Zones p. 112). In locations where supersaturation occurs, the increased levels of oxygen should conform to the recommendations in the discussion of Total Dissolved Gases, p. 139.

TOTAL DISSOLVED GASES (SUPERSATURATION)

Excessive total dissolved gas pressure (supersaturation) is a relatively new aspect of water quality. Previously, supersaturation was believed to be a problem that was limited to the water supplies of fish culture facilities (Shelford and Allee 1913).[135] Lindroth (1957)[126] reported that spillways at hydroelectric dams in Sweden caused supersaturation, and recently Ebel (1969)[112] and Beiningen and Ebel (1968)[103] established that spillways at dams caused gas bubble disease to be a limiting factor for aquatic life in the Columbia and Snake Rivers. Renfro (1963)[133] and others reported that excessive algal blooms have caused gas bubble disease in lentic water. DeMont and Miller (*in press*)[110] and Malous et al. (1972)[127] reported gas bubble disease among fish and mollusks living in the heated effluents of steam generating stations. Therefore, modified dissolved gas pressures as a result of dams, eutrophication, and thermal discharges present a widespread potential for adversely affecting fish and aquatic invertebrates. Gas bubble disease has been studied frequently since Gorham (1898,[119] 1899[120]) published his initial papers, with the result that general knowledge of the causes, consequences, and adverse levels are adequate to evaluate criteria for this water quality characteristic.

Gas bubble disease is caused by excessive total dissolved gas pressure *but it is not caused by the dissolved nitrogen gas alone* (Marsh and Gorham 1904,[128] Shelford and Allee 1913,[135] Englehorn 1943,[115] Harvey et al. 1944a,[121] Doudoroff 1957,[111] Harvey and Cooper 1962).[123] Englehorn (1943)[115] analyzed the gases contained in the bubbles that were formed in fish suffering from gas bubble disease and found that their gas composition was essentially identical to air. This was confirmed by Shirahata (1966).[136]

Etiologic Factors

Gas bubble disease (GBD) results when the uncompensated total gas pressure is greater in the water than in the air, but several important factors influence the etiology of GBD. These factors include: exposure time and physical factors such as hydrostatic pressure; other compensating forces and biological factors such as species or life stage tolerance or levels of activity; and any other factors that influence gas solubility. Of these factors perhaps none are more commonly misunderstood than the physical roles of total dissolved gas pressure* and hydrostatic pressure. The following discussion is intended to clarify these roles.

Each component gas in air exerts a measurable pressure, and the sum of these pressures constitutes atmospheric or barometric pressure, which is equivalent per unit of surface area at standard conditions to a pressure exerted by a column of mercury 760mm high or a column of water about 10 meters high (at sea level, excluding water vapor pressure). The pressure of an individual gas in air is called a *partial pressure*, and in water it is called a *tension*; both terms are an acknowledgement that the pressure of an individual gas is only part of the total atmospheric pressure. Likewise, each component gas will dissolve in water independently of all other gases, and when at equilibrium with the air, the pressure (tension) of a specific dissolved gas is equivalent to its partial pressure in the air. This relationship is evident in Table III-5 which lists the main constituents of dry air and their approximate partial pressures at sea level.

When supersaturation occurs, the diffusion pressure imbalance between the dissolved gas phase and the atmospheric phase favors a net transfer of gases from the water to the air. Generally this transfer cannot be accomplished fast enough by diffusion alone to prevent the formation of gas bubbles. However, a gas bubble cannot form in the water unless gas nuclei are present (Evans and Walder 1969,[116] Harvey et al. 1944b[122]) and unless the total dissolved gas pressure exceeds the sum of the compensating pressures such as hydrostatic pressure. Additional compensating pressures include blood pressure and viscosity, and their benefits may be significant.

Gas nuclei are probably unavoidable in surface water or in animals, because such nuclei are generated by any factor which decreases gas solubility, and because extreme measures are required to dissolve gas nuclei (Evans and Walder 1969;[116] Harvey et al. 1944b).[122] Therefore, hydrostatic pressure is a major preventive factor in gas bubble disease.

The effect of hydrostatic pressure is to oppose gas bubble formation. For example, one cannot blow a bubble out of a tube immersed in water until the gas pressure in the tube slightly exceeds the hydrostatic pressure at the end of the tube. Likewise a bubble cannot form in water, blood, or

TABLE III-5—Composition of Dry Air and Partial Pressures of Selected Gases at Sea Level

Gas	Molecular[a] percentage in dry air	Times atmospheric pressure	Individual gas[b] pressure in air or water at sea level
N_2	78.084	×760 mm Hg	=593.438 mm Hg
O_2	20.946	"	159.189 "
Ar	0.[illegible]	"	7.098 "
CO_2	0.033	"	0.250 "
Ne	0.00181	"	0.0138 "
He	0.00052	"	0.0039 "
			759.9927 mm Hg

[a] Glueckauf (1951[118]).

[b] At standard conditions excluding corrections for water vapor pressure.

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* In this Section gas tension will be called gas pressure and total gas tension will be called total dissolved gas pressure (TDGP). This is being done as a descriptive aid to readers who are not familiar with the terminology and yet need to convey these principles to laymen.

tissue until the total gas pressure therein exceeds the sum of atmospheric pressure (760mm Hg) plus hydrostatic pressure plus any other restraining forces. This relationship is illustrated in Figure III-1 which shows, for example, that gas bubbles could form in fresh water to a depth of about one meter when the total dissolved gas pressure is equal to 1.10 atmospheres; but they could not form below that point.

Excessive total dissolved gas pressure relative to ambient atmospheric pressure, therefore, represents a greater threat to aquatic organisms in the shallow but importantly productive littoral zone than in the deeper sublittoral zone. For example, if fish or their food organisms remain within a meter of the surface in water having a total dissolved gas pressure of 1.10 atmospheres, they are theoretically capable of developing gas bubble disease, especially if their body processes further decrease gas solubility by such means as physical activity, metabolic heat, increased osmolarity, or decreased blood pressure.

Hydrostatic pressure only opposes bubble formation; it does not decrease the kinetic energy of dissolved gas molecules except at extreme pressures. If this were not the case, aerobic animal life would be eliminated at or below a water depth equivalent to the pressure of oxygen, because there would be no oxygen pressure to drive O_2 across the gill membrane and thence into the blood. For a more detailed discussion of this subject, the reader is referred to Van Liere and Stickney's (1963)[138] and Randall's (1970a)[131] excellent reviews.

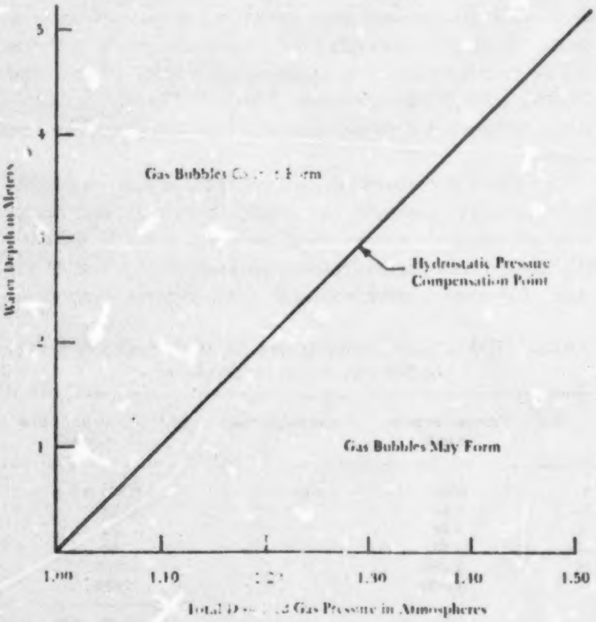

FIGURE III-1—Relationship of Total Dissolved Gas Pressure to Hydrostatic Pressure in Preventing Gas Bubble Formation

A final example will clarify the importance of total dissolved gas pressure. Eutrophic lakes often become supersaturated with photosynthetic dissolved oxygen, and such lakes commonly approach (or exceed) 120 per cent of saturation values for oxygen. But this only represents an additional dissolved gas pressure of about 32mm Hg ($O_2 = 159.19$ mm Hg$\times0.2 = 31.83$ mm Hg) which equals:

$$\frac{760 \text{ mm Hg} + 31.83 \text{ mm Hg}}{760 \text{ mm Hg}} = 1.041 \text{ atmospheres of total dissolved gas pressure}$$

This imbalance apparently can be compensated in part by metabolic oxygen consumption, blood pressure, or both. On the other hand, a 1,000-fold increase in the neon saturation level would only increase the total dissolved gas pressure by about 1.8 mm Hg or:

$$\frac{1.8 \text{ mm Hg} + 760 \text{ mm Hg}}{760 \text{ mm Hg}} = 1.002 \text{ atmospheres}$$

This would not cause gas bubble disease.

The opposite situation can occur in spring water, where dissolved oxygen pressure is low and dissolved nitrogen and other gas pressures are high. In an actual case (Schneider *personal communication*),[144] dissolved nitrogen was reported to be 124 per cent of its air saturation value, whereas oxygen was 46 per cent of its air saturation value; total gas pressure was 1.046 of dry atmospheric pressure. Fish were living in this water, and although they probably suffered from hypoxia, they showed no symptoms of gas bubble disease.

How dissolved gases come out of solution and form bubbles (cavitate) is a basic physical and physiological topic which is only summarized here. Harvey et al. (1944b)[122] determined that bubble formation is promoted by boundary zone or surface interfaces which reduce surface tension and thereby decrease the dissolved gas pressure required for cavitation. For this reason, one usually sees gas bubbles forming first and growing fastest on submersed interfaces, such as tank walls, sticks, or the external surfaces of aquatic life.

Gas nuclei are apparently required for bubble formation, and these are considered to be ultra micro bubbles (Evans and Walder 1969).[116] These nuclei apparently represent an equilibrium between the extremely high compressive energy of surface tension and the pressure of contained gases. Lack of gas nuclei probably accounts for instances when extremely high but uncompensated dissolved gas pressures failed to cause bubble formation (Pease and Blinks 1947,[130] Hemmingsen 1970).[124] Gas nuclei are produced by anything that decreases gas solubility or surface tension (Harvey et al. 1944b,[122] Hills 1967,[125] Evans and Walder 1969)[116] and they can be eliminated at least temperarily by extremely high pressure which drives them back into solution (Evans and Walder 1969).[116]

Possible causes of gas nuclei formation in organisms include negative pressures in skeletal or cardiac muscle during pronounced activity (Whitaker et al. 1945),[141] eddy currents in the blood vascular system, synthetic or biologically produced surface-active compounds, and possible salting-out effects during hemoconcentration (as in saltwater adaptation). Once a bubble has formed, it grows via the diffusion of all gases into it.

Many factors influence the incidence and severity of gas bubble disease. For example, the fat content of an animal may influence its susceptibility. This has not been studied in fish, but Boycott and Damant (1908),[106] Behnke (1942),[102] and Gersh et al. (1944)[117] report that fat mammals are more susceptible than lean mammals to the "bends" in high-altitude decompression. This may be particularly significant to non-feeding adult Pacific salmon which begin their spawning run with considerable stored fat. This may also account in part for differences in the tolerances of different age groups or fish species. Susceptibility to gas bubble disease is unpredictable among wild fish, particularly when they are free to change their water depth and level of activity.

Gas Bubble Disease Syndrome and Effects

Although the literature documents many occurrences of gas bubble disease, data are usually missing for several important physical factors, such as hydrostatic pressure, barometric pressure, relative humidity, salinity, temperature, or other factors leading to calculation of total dissolved gas pressure. The most frequently reported parameter has been the calculated dissolved nitrogen (N_2) concentration or its percentage saturation from which one can estimate the pressure of inert gases. Thus the reported N_2 values provide only a general indication of the total dissolved gas pressure, which unfortunately tends to convey the erroneous concept that N_2 is the instigative or only significant factor in gas bubble disease.

Gas bubbles probably form first on the external surfaces of aquatic life, where total hydrostatic pressure is least and where an interface exists. Bubbles within the body of animals probably form later at low dissolved gas pressures, because blood pressure and other factors may provide additional resistance to bubble formation. However, at high dissolved gas pressure (>1.25 atm) bubbles in the blood may be the first recognizable symptom (Schneider *personal communication*).[144] In the case of larval fishes, zooplankton, or other small forms of aquatic life, the effect of external bubbles may be a blockage of the flow of water across the gills and asphyxiation or a change in buoyancy (Shirahata 1966).[136] The latter probably causes additional energy expenditure or floatation, causing potentially lethal exposure to ultraviolet radiation or potential predation.

The direct internal effects of gas bubble disease include a variety of symptoms that appear to be related primarily to the level of total dissolved gas pressure, the exposure time, and the *in vivo* location of lowest compensatory pressure.

The following is a résumé of Shirahata's (1966)[136] results. As the uncompensated total dissolved gas pressure increases, bubbles begin to appear on the fish, then within the skin, the roof of the mouth, within the fins, or within the abdominal cavity. Gas pockets may also form behind the eyeball and cause an exophthalmic "pop-eyed" condition. Probably gas emboli in the blood are the last primary symptoms to develop, because blood pressure and plasma viscosity oppose bubble formation. At some as yet undefined point, gas emboli become sufficiently large and frequent to cause hemostasis in blood vessels, which in turn may cause extensive tissue damage or complete hemostasis by filling the heart chamber with gas. The latter is the usual direct cause of death.

Exophthalmus or "pop-eye" and eye damage can be caused by several factors other than gas bubble disease and one should be duly cautious when tempted to diagnose gas bubble disease based solely on these criteria. While the above symptoms can be caused by excessive dissolved gas pressure (Westgard 1964),[140] they can also be caused by malnutrition, abrasion, and possibly by infection. Unfortunately there is no known definitive way to distinguish between latent eye damage caused by previous exposure to excessive dissolved gas pressure and other causes.

Secondary, latent, or sublethal effects of gas bubble disease in fish include promoting other diseases, necrosis, or other tissue changes, hemorrhages, blindness, and reproductive failure (Harvey and Cooper 1962,[123] Westgard 1964,[140] Pauley and Nakatani 1967,[133] and Bouck et al. 1971).[105] There is no known evidence that supersaturation causes a nitrogen narcosis in fish (such as can be experienced by scuba divers), as this requires high dissolved gas pressures probably above 10 atm. However, one can expect that fish afflicted with gas bubble disease or the above secondary effects might have their normal behavior altered.

There is no definitive evidence that fishes can detect supersaturation (Shelford and Allee 1913,[135] or that they actively avoid it by seeking hydrostatic pressure compensation (Ebel 1969).[112] However, the potential capacity to avoid supersaturation or to compensate by sounding is limited among anadromous species by the necessity of ascending their home river and by dams with relatively shallow fish ladders. This may also apply to other species that reproduce in or otherwise live in shallow-water niches. Physiological adaptation to supersaturation seems unlikely, and this contention is supported by the preliminary studies of Coutant and Genoway (1968).[109]

Interaction between gas bubble disease and other stresses is highly likely but not clearly established. Fish were more susceptible to a given level of total dissolved gas pressure when wounded (Egusa 1955).[114] The thermal tolerance of Pacific salmon was reduced when N_2 levels were 125 to 180 per cent in the case of juveniles (Ebel et al. 1971),[113] and when N_2 levels were >118 per cent in the case of adults (Coutant and Genoway 1968).[109] Chemicals or other factors that influence body activity or cardiovascular activity may also influence blood pressure (Randall 1970b),[132] and this would be expected to influence the degree to which the dissolved gas pressure is in excess, and hence the tolerance to gas bubble disease.

Variation in biological response is a prominent aspect of gas bubble disease, which should not be surprising in view of the numerous influential factors. Some of this variation might be explained by physiological differences between life stages or species, degree of fatness, blood pressure, blood viscosity, metabolic heat, body size, muscular activity, and blood osmolarity. For example, susceptibility to gas bubble disease may be inversely related to blood (or hemolymph) pressure. There is wide variation in blood pressure between life stages, between fish species, and between invertebrate species. Based on aortic blood pressures alone, one can hypothesize that largemouth bass (*Micropterus salmoides*) might be more susceptible to gas bubble disease than chinook salmon (*Oncorhynchus tshawytscha*) if other factors are equal. This contention is also supported by the observations that gas bubbles form in the blood of bullfrogs more easily than in rats (Berg et al. 1945),[104] possibly because of differences in blood pressure (Brand et al. 1951).[107]

Tolerance to supersaturation also varies between body sizes or life stages; Shirahata (1966)[136] relates this, in part, to an increase in cardiac and skeletal muscle activity. Larger fish were generally more sensitive to supersaturation than were smaller fish in most studies (Wiebe and McGavock 1932,[142] Egusa 1955,[114] Shirahata 1966,[136] Harvey and Cooper 1962).[123] Wood (1968)[143] has the opposite view, but he provides no supporting evidence. Possibly larger fish are more susceptible to gas bubble disease in part because they can develop greater metabolic heat than smaller fish. In this regard, Carey and Teal (1969)[108] reported that large tuna may have a muscle temperature as much as 10 C above the water temperature.

Data are quite limited on the tolerance of zooplankters and other aquatic invertebrates to excessive dissolved gas pressure. Evans and Walder (1969)[116] demonstrated that invertebrates can develop gas bubble disease. Unpublished observations by Nebeker* demonstrate that *Daphnia* sp. and *Gammarus* sp. are susceptible to gas bubble disease. On the other hand, it is widely known that some aquatic invertebrates are capable of diel migrations that may expose them to a considerable change in dissolved gas pressure; but apparently these organisms can tolerate or otherwise handle such changes. In view of the paucity of data, nothing firm can be said regarding the general tolerance of invertebrates to supersaturation.

Analytical Considerations

The apparatus and method of Van Slyke et al. (1934)[133] are still the standard analytical tools for most gas analyses. Scholander et al. (1955)[134] and others have developed similar methods with modifications to accomodate their special needs. More recently, Swinnerton et al. (1962)[137] published a gas analysis method that utilizes gas-liquid chromatography. However, both of these basic methods have drawbacks, because they either require special expertise or do not otherwise meet the field needs of limnologists and fisheries or pollution biologists.

A new device by Weiss* measures the differential gas pressure between the air and the water within fifteen minutes. This portable device is simple to operate, easy and inexpensive to build, and gives direct readings in mm Hg. Unpublished data by Weiss show that this instrument has an accuracy comparable to the Van Slyke and the chromatographic procedures. The instrument consists of a gas sensor (150 ft. coil of small diameter, thin-walled, silicone rubber tube) connected to a mercury manometer. The sensor is placed underwater where the air in the tubing equilibrates with the dissolved gases in the water. The resulting gas pressure is read directly via the mercury manometer which gives a positive value for supersaturated water and a negative value for water that is not fully saturated.

Total Dissolved Gas Pressure Criteria

Safe upper limits for dissolved gases must be based on the total dissolved gas pressures (sum of all gas tensions) and not solely on the saturation value of dissolved nitrogen gas alone. Furthermore, such limits must provide for the safety of aquatic organisms that inhabit or frequent the shallow littoral zone, where an existing supersaturation could be worsened by heating, photosynthetic oxygen production, or other factors. There is little information on the chronic sublethal effects of gas bubble disease and almost all the research has been limited to species of the family Salmonidae. Likewise, gas tolerance data are unavailable for zooplankters and most other aquatic invertebrates. Therefore, it is necessary to judge safe limits from data on mortality of selected salmonid fishes that were held under conditions approximating the shallow water of a hypothetical littoral zone. These data are:

1. Shirahata (1966)[136] reported that advanced fry of rainbow trout (*Salmo gairdneri*) experienced 10 per cent mortality when N_2 was about 111 per cent of its saturation value. He concludes that, "... the nitrogen contents which did not cause any gas disease were ... less than 110 per cent to the more advanced fry."

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2. Harvey and Cooper (1962)[122] reported that fry of sockeye salmon (*Oncorhynchus nerka*) suffered latent effects (necrosis and hemorrhages) for some time after normal gas levels were said to have been restored.

3. Coutant and Genoway (1968)[109] reported that sexually precocious spring chinook salmon (*Oncorhynchus tshawytscha*) weighing 2 to 4 kg, experienced extensive mortality in six days when exposed at or above 118 per cent of N_2 saturation; these salmon experienced no mortality when N_2 was below 110 per cent of saturation.

Whether or not other species or life stages of aquatic life may be more or less sensitive than the above salmonids remains to be proven. In the meantime, the above references provide the main basis for establishing the following total dissolved gas recommendations.

Recommendations

Available data for salmonid fish suggest that aquatic life will be protected only when *total* dissolved gas pressure in water is no greater than 110 per cent of the existing atmospheric pressure. Any prolonged artificial increase in total dissolved gas pressure should be avoided in view of the incomplete body of information.

CARBON DIOXIDE

Carbon dioxide exists in two major forms in water. It may enter into the bicarbonate buffering system at various concentrations depending on the pH of the water. In addition, "free" carbon dioxide may also exist, and this component affects the respiration of fish (Fry 1957).[151] Because of respiratory effects, free carbon dioxide is the form considered most significant to aquatic life.

The concentration of free carbon dioxide, where oxygen-demanding wastes are not excessive, is a function of pH, temperature, alkalinity, and the atmospheric pressure of carbon dioxide. Doudoroff (1957)[147] reported that concentrations of free carbon dioxide above 20 mg/l occur rarely, even in polluted waters; and Ellis (1937)[150] found that the free carbon dioxide content of Atlantic Coast streams ranged between zero and 12 mg/l. Ellis (1937)[150] and Hart (1944)[152] both reported that in 90 to 95 per cent of the fresh waters in the United States that support a good and diverse fish population the free carbon dioxide concentrations fall below 5 mg/l.

An excess of free carbon dioxide may have adverse effects on aquatic life. Powers and Clark (1943)[156] and Warren (1971)[157] reported that fish are able to detect and to respond to slight gradients in carbon dioxide tension. Brinley (1943)[146] and Höglund (1961)[154] observed that fish may avoid free carbon dioxide levels as low as 1.0 to 6.0 mg/l.

Elevated carbon dioxide concentrations may interfere with the ability of fish to respire properly and may thus affect dissolved oxygen uptake. Doudoroff and Katz (1950)[148] and Doudoroff and Shumway (1970)[149] reported that where dissolved oxygen uptake interference does occur, the free carbon dioxide concentrations which appreciably affect this are higher than those found in polluted waters. In bioassay tests using ten species of warmwater fish, Hart (1944)[152] found that the gizzard shad (*Dorosoma cepedianum*) was the most sensitive and was unable to remove oxygen from water 50 per cent saturated with dissolved oxygen in the presence of 88 mg/l of free carbon dioxide. The less sensitive, largemouth bass (*Micropterus salmoides*) was unable to extract oxygen when the carbon dioxide level reached 175 mg/l. Below 60 mg/l of free carbon dioxide, most species of fish had little trouble in extracting dissolved oxygen from the water.

High concentrations of free carbon dioxide cause pronounced increases in the minimum dissolved oxygen requirement of coho salmon (*Oncorhynchus kisutch*), but these fish acclimatized rapidly to carbon dioxide concentrations as high as 175 mg/l at 20 C when the dissolved oxygen level was near saturation (McNeil 1956).[155]

Basu (1959)[145] found that for most fish species, carbon dioxide affected the fishes' ability to consume oxygen in a predictable manner. He further indicated that temperature affected carbon dioxide sensitivity, being less at higher water temperatures.

The ability of fish to acclimatize to increases in carbon dioxide concentrations as high as 60 mg/l with little effect has been indicated by Haskell and Davies (1958).[153] Doudoroff and Shumway (1970)[149] indicate that the ability of fish to detect low free carbon dioxide concentrations, the presence of low carbon dioxide levels in most waters, and the ability of fish to acclimatize to carbon dioxide in the water probably prevent this constituent from becoming a major hazard.

Recommendation

Concentrations of free carbon dioxide above 20 mg/l occur rarely. Fish acclimatize to increases in carbon dioxide levels as high as 60 mg/l with little effect. However, fish are able to detect and respond to slight gradients and many avoid free carbon dioxide levels as low as 1.0 to 6.0 mg/l.

ACIDITY, ALKALINITY, AND pH

NATURAL CONDITIONS AND SIGNIFICANCE

Acidity in natural waters is caused by carbon dioxide, mineral acids, weakly dissociated acids, and the salts of strong acids and weak bases. The alkalinity of a water is actually a measure of the capacity of the carbonate-bicarbonate system to buffer the water against change in pH. Technical information on alkalinity has recently been reviewed by Kemp (1971).[162]

An index of the hydrogen ion activity is pH. Even though pH determinations are used as an indication of acidity or alkalinity or both, pH is not a measure of either. There is a relationship between pH, acidity, and alkalinity (Standard Methods 1971):[164] water with a pH of 4.5 or lower has no measurable alkalinity, and water with a pH of 8.3 or higher has no measurable acidity. In natural water, where the pH may often be in the vicinity of 8.3, acidity is not a factor of concern. In most productive fresh waters, the pH falls in a range between 6.5 and 8.5 (except when increased by photosynthetic activity). Some regions have soft waters with poor buffering capacity and naturally low pH. They tend to be less productive. Such conditions are found especially in dark colored waters draining from coniferous forests or muskegs, and in swampy sections of the Southeast. For a variety of reasons, some waters may exhibit quite extreme pH values. Before these are considered natural conditions, it should be ascertained that they have not actually resulted from man-made changes, such as stripping of ground cover or old mining activities. This is important because the recommendations refer to estimated natural levels.

TOXICITY TO AQUATIC LIFE

Some aquatic organisms, especially algae, have been found to live at pH 2 and lower, and others at pH 10 and higher; however, such organisms are relatively few. Some natural waters with a pH of 4 support healthy populations of fish and other organisms. In these cases the acidity is due primarily to carbon dioxide and natural organic acids, and the water has little buffering capacity. Other natural waters with a pH of 9.5 also support fish but are not usually highly productive.

The effects of pH on aquatic life have been reviewed in detail in excellent reports by the European Inland Fisheries Advisory Commission (1969)[160] and Katz (1969).[151] Interpretations and summaries of these reviews are given in Table III-6.

ADVERSE INDIRECT EFFECTS OR SIDE EFFECTS

Addition of either acids or alkalies to water may be harmful not only by producing acid or alkaline conditions, but also by increasing the toxicity of various components in the waters. For example, acidification of water may release free carbon dioxide. This exerts a toxic action additional to that of the lower pH. Recommendations for pH are valid if carbon dioxide is less than 25 mg/l (see the discussion of Carbon Dioxide, p. 139).

A reduction of about 1.5 pH units can cause a thousand-fold increase in the acute toxicity of a metallocyanide complex (Doudoroff et al. 1966).[159] The addition of strong alkalies may cause the formation of undissociated NH_4OH or un-ionized NH_3 in quantities that may be toxic (Lloyd 1961,[163] Burrows 1964).[155] Many other pollutants may change their toxicity to a lesser extent. It is difficult to predict whether toxicity will increase or decrease for a given direction of change in pH.

Weakly dissociated acids and bases must be considered in terms of their toxicities, as well as their effects on pH and alkalinity.

The availability of many nutrient substances varies with the hydrogen ion concentration. Some trace metals become more soluble at low pH. At higher pH values, iron tends to become unavailable to some plants, and hence the production of the whole aquatic community may be affected.

The major buffering system in natural waters is the carbonate system that not only neutralizes acids and bases to reduce the fluctuations in pH, but also forms a reservoir of carbon for photosynthesis. This process is indispensable, because there is a limit on the rate at which carbon dioxide can be obtained from the atmosphere to replace that in the water. Thus the productivity of waters is closely correlated to the carbonate buffering system. The addition of mineral acids preempts the carbonate buffering capacity, and the

TABLE III–6—*A Summary of Some Effects of pH on Freshwater Fish and Other Aquatic Organisms*

pH	Known effects
11.5–12.0	Some caddis flies (Trichoptera) survive but emergence reduced.
11.0–11.5	Rapidly lethal to all species of fish.
10.5–11.0	Rapidly lethal to salmonids. The upper limit is lethal to carp (Cyprinus carpio), goldfish (Carassius auratus), and pike. Lethal to some stoneflies (Plecoptera) and dragonflies (Odonata). Caddis fly emergence reduced.
10.0–10.5	Withstood by salmonids for short periods but eventually lethal. Exceeds tolerance of bluegills (Lepomis macrochirus) and probably goldfish. Some typical stoneflies and mayflies (Ephemera) survive with reduced emergence.
9.5–10.0	Lethal to salmonids over a prolonged period of time and no viable fishery for coldwater species. Reduces populations of warmwater fish and may be harmful to development stages. Causes reduced emergence of some stoneflies.
9.0–9.5	Likely to be harmful to salmonids and perch (Perca) if present for a considerable length of time and no viable fishery for coldwater species. Reduced populations of warmwater fish. Carp avoid these levels.
8.5–9.0	Approaches tolerance limit of some salmonids, whitefish (Coregonus), catfish (Ictaluridae), and perch. Avoided by goldfish. No apparent effects on invertebrates.
8.0–8.5	Motility of carp sperm reduced. Partial mortality of burbot (Lota lota) eggs.
7.0–8.0	Full fish production. No known harmful effects on adult or immature fish, but 7.0 is near low limit for Gammarus reproduction and perhaps for some other crustaceans.
6.5–7.0	Not lethal to fish unless heavy metals or cyanides that are more toxic at low pH are present. Generally full fish production, but for fathead minnow (Pimephales promelas), frequency of spawning and number of eggs are somewhat reduced. Invertebrates except crustaceans relatively normal, including common occurrence of mollusks. Microorganisms, algae, and higher plants essentially normal.
6.0–6.5	Unlikely to be toxic to fish unless free carbon dioxide is present in excess of 100 ppm. Good aquatic populations with varied species can exist with some exceptions. Reproduction of Gammarus and Daphnia prevented, perhaps other crustaceans. Aquatic plants and microorganisms relatively normal except fungi frequent.
5.5–6.0	Eastern brook trout (Salvelinus fontinalis) survive at over pH 5.5. Rainbow trout (Salmo gairdneri) do not occur. In natural situations, small populations of relatively few species of fish can be found. Growth rate of carp reduced. Spawning of fathead minnow significantly reduced. Mollusks rare.
5.0–5.5	Very restricted fish populations but not lethal to any fish species unless CO_2 is high (over 25 ppm), or water contains iron salts. May be lethal to eggs and larvae of sensitive fish species. Prevents spawning of fathead minnow. Benthic invertebrates moderately diverse, with certain black flies (Simuliidae), mayflies (Ephemerella), stoneflies, and midges (Chironomidae) present in numbers. Lethal to other invertebrates such as the mayfly. Bacterial species diversity decreased; yeasts and sulfur and iron bacteria (Thiobacillus-Ferrobacillus) common. Algae reasonably diverse and higher plants will grow.
4.5–5.0	No viable fishery can be maintained. Likely to be lethal to eggs and fry of salmonids. A salmonid population could not reproduce. Harmful, but not necessarily lethal to carp. Adult brown trout (Salmo trutta) can survive in peat waters. Benthic fauna restricted, mayflies reduced. Lethal to several typical stoneflies. Inhibits emergence of certain caddis fly, stonefly, and midge larvae. Diatoms are dominant algae.
4.0–4.5	Fish populations limited: only a few species survive. Perch, some coarse fish, and pike can acclimate to this pH, but only pike reproduce. Lethal to fathead minnow. Some caddis flies and dragonflies found in such habitats; certain midges dominant. Flora restricted.
3.5–4.0	Lethal to salmonids and bluegills. Limit of tolerance of pumpkinseed (Lepomis gibbosus), perch, pike, and some coarse fish. All flora and fauna severely restricted in number of species. Cattail (Typha) is only common higher plant.
3.0–3.5	Unlikely that any fish can survive for more than a few hours. A few kinds of invertebrates such as certain midges and alderflies, and a few species of algae may be found at this pH range and lower

original biological productivity is reduced in proportion to the degree that such capacity is exhausted. Therefore, the minimum essential buffering capacity and tolerable pH limits are important water quality considerations.

Because of this importance, there should be no serious depletion of the carbonate buffering capacity, and it is recommended that reduction of alkalinity of natural waters should not exceed 25 per cent.

Recommendations

Suggested maximum and minimum levels of protection for aquatic life are given in the following recommendations. A single range of values could not apply to all kinds of fish, nor could it cover the different degrees of graded effects. The selection of the level of protection is a socioeconomic decision, not a biological one. The levels are defined in Table III–3 (see the discussion of Dissolved Oxygen).

Nearly Maximum Level of Protection

- **pH not less than 6.5 nor more than 8.5. No change greater than 0.5 units above the estimated natural seasonal maximum, nor below the estimated natural seasonal minimum.**

High Level of Protection

- **pH not less than 6.0 nor more than 9.0. No change greater than 0.5 units outside the estimated natural seasonal maximum and minimum.**

Moderate Level of Protection

- **pH not less than 6.0 nor more than 9.0. No change greater than 1.0 units outside the estimated natural seasonal maximum and minimum.**

Low Level of Protection

- **pH not less than 5.5 nor more than 9.5. No change greater than 1.5 units outside the estimated natural seasonal maximum and minimum.**

Additional Requirements for All Levels of Protection

- **If a natural pH is outside the stated range of pH for a given level of protection, no further change is desirable.**
- **The extreme range of pH fluctuation in any location should not be greater than 2.0 units. If natural fluctuation exceeds this, pH should not be altered.**
- **The natural daily and seasonal patterns of pH variation should be maintained, although the absolute values may be altered within the limits specified.**
- **The total alkalinity of water is not to be decreased more than 25 per cent below the natural level.**

DISSOLVED SOLIDS AND HARDNESS

Surface water at some time and place may contain a trace or more of any water-soluble substance. The significance and the effects of small concentrations of these substances are discussed separately throughout this Report. The presence and relative abundance of these constituents in water is influenced by several factors, including surface runoff, geochemistry of the watershed, atmospheric fallout including snow and rainfall, man-created effluents, and biological and chemical processes in the water itself. Many of these dissolved materials are essential to the life processes of aquatic organisms. For a general discussion of the chemistry of fresh water the reader is referred to Hutchinson (1957)[167] and Ruttner (1963).[172]

A general term describing the concentration of dissolved materials in water is *total dissolved solids*. The more conspicuous constituents of total dissolved solids in natural surface waters include carbonates, sulfates, chlorides, phosphates, and nitrates. These anions occur in combination with such metallic cations as calcium, sodium, potassium, magnesium, and iron to form ionizable salts (Reid 1961).[170]

Concentrations and relative proportions of dissolved materials vary widely with locality and time. Hart et al. (1945)[164] reported that in the inland waters of the United States which support a mixed biota, 5 per cent have a dissolved solids concentration under 72 mg/l; about 50 per cent under 169 mg/l; and 95 per cent under 400 mg/l. Table III-7 provides information on ranges and median concentrations of the major ions in United States streams.

The quantity and quality of dissolved solids are major factors in determining the variety and abundance of plants and animals in an aquatic system. They serve as nutrients in productivity, osmotic stress, and direct toxicity. A major change in quantity or composition of total dissolved solids changes the structure and function of aquatic ecosystems. Such changes are difficult to predict.

Concentrations of dissolved solids affecting freshwater fish by osmotic stress are not well known. Mace (1953)[169] and Rounsefell and Everhart (1953)[171] reported that the upper limit may range between 5,000 and 10,000 mg/l total dissolved solids, depending on species and prior acclimatization. The literature indicates that concentrations of total dissolved solids that cause osmotic stress in adult fish are higher than the concentrations existing in most fresh waters of the United States. Many dissolved materials are toxic at concentrations lower than those where osmotic effect can be expected. (See Toxic Substances, p. 172, and Acidity, Alkalinity, and pH, p. 140.)

Hardness of surface waters is a component of total dissolved solids and is chiefly attributable to calcium and magnesium ions. Other ions such as strontium, barium, manganese, iron, copper, zinc, and lead add to hardness, but since they are normally present in minor concentrations their effect is usually minimal. Generally, the biological productivity of a water is directly correlated with its hardness. However, while calcium and magnesium contribute to hardness and productivity, many other elements (when present in concentrations which contribute a substantial measure of hardness) reduce biological productivity and are toxic. Hardness per se has no biological significance because biological effects are a function of the specific concentrations and combinations of the elements present.

The term "hardness" serves a useful purpose as a general index of water type, buffering capacity, and productivity. Waters high in calcium and magnesium ions (hard water) lower the toxicity of many metals to aquatic life (Brown 1968;[165] Lloyd and Herbert 1962).[168] (See Figure III-9 in the discussion of Metals, p. 178.) However, the term "hardness" should be avoided in delineating water quality

TABLE III-7—Major Dissolved Constituents of River Waters Representing About 90 Percent of Total Stream Flow in the United States

Constituent	Median mg/l	Range mg/l
Total dissolved solids	169	72–400
Bicarbonate (HCO_3)	90	40–180
Sulfate (SO_4)	32	11–90
Chloride (Cl)	9	3–173
Calcium (Ca)	28	15–52
Magnesium (Mg)	7	3.5–14
Sodium and potassium (Na and K)	10	6–85

Source: After Hart et al. (1945)[164]

requirements for aquatic life. More emphasis should be placed on specific ions.

Recommendation

Total dissolved materials should not be changed to the extent that the biological communities characteristic of particular habitats are significantly changed. When dissolved materials are altered, bioassays and field studies can determine the limits that may be tolerated without endangering the structure and function of the aquatic ecosystem.

OILS

Losses of oil that can have an adverse effect on water quality and aquatic life can occur in many of the phases of oil production, refining, transportation, and use. Pollution may be in the form of floating oils, emulsified oils, or solution of the water soluble fraction of these oils.

The toxicity of crude oil has been difficult to interpret since crude oil may contain many different organic compounds and inorganic elements. The composition of such oils may vary from region to region, and petroleum products produced can be drastically different in character in line with their different intended uses (Purdy 1958).[198] The major components of crude oil can be categorized as aliphatic normal hydrocarbons, cyclic paraffin hydrocarbons, aromatic hydrocarbons, naphtheno-aromatic hydrocarbons, resins, asphaltenes, heteroatomic compounds, and metallic compounds (Bestougeff 1967).[175] The aromatic hydrocarbons in crude oil appear to be the major group of acutely toxic compounds (Blumer 1971,[176] Shelton 1971).[199]

Because the biological effects of oils and the relative merits of control measures are discussed in detail in Section IV (p. 257) of this Report, only effects of special interest or pertinence to fresh water are discussed here. The effects of floating oil on wildlife are discussed on p. 196.

OIL REFINERY EFFLUENTS

Copeland and Dorris (1964)[180] studied primary productivity and community respiration in a series of oil refinery effluent oxidation ponds. These ponds received waste waters which had been in contact with the crude oil and various products produced within the refinery. Surface oils were skimmed. In the series of oxidation ponds, primary productivity and community respiration measurements clearly indicated that primary producers were limited in the first ponds, probably by toxins in the water. Oxidation ponds further along in the series typically supported algal blooms. Apparently degradation of the toxic organic compounds reduced their concentration below the threshold lethal to the algae. Primary productivity was not greater than community respiration in the first ponds in the series. Minter (1964)[195] found that species diversity of phytoplankton was lowest in the first four ponds of the series of ten. A "slug" of unknown toxic substance drastically reduced the species diversity in all ponds. Zooplankton volumes increased in the latter half of the pond series, presumably as a result of decreasing toxicity. Benthic fauna species diversity in streams receiving oil refinery effluents was low near the outfall and progressively increased downstream as biological assimilation reduced the concentration of toxins (Wilhm and Dorris 1966,[206] Harrel et al. 1967,[183] Mathis and Dorris 1968[191]).

Long-term, continuous-flow bioassays of biologically treated oil refinery effluents indicated that complex refineries produce effluents which contain cumulative toxins of substances that cause accumulative deleterious effects (Graham and Dorris 1968).[182] Subsequent long-term continuous-flow bioassays of biologically treated oil refinery effluents indicated that passage of the effluent through activated carbon columns does not remove the fish toxicants. Of the fathead minnows (*Pimphales promelas*) tested, half were killed in 14 days, and only 10 per cent survived 30 days (Burks 1972[207] *personal communication*). Trace organic compounds identified in extracts from the effluent were a homologous series of aliphatic hydrocarbons ($C_{11}H_{24}$ through $C_{15}H_{32}$) and isomers of cresol and xylenol. Since the soluble fractions derived from oil refineries are quantitatively, and to some extent qualitatively, different from those derived from oil spills, care must be taken to differentiate between these two sources.

FREE AND FLOATING OIL

Free oil or emulsions may adhere to the gills of fish, interfering with respiration and causing asphyxia. Within limits, fish are able to combat this by defensive mucous secretions (Cole 1941).[179] Free oil and emulsions may likewise coat aquatic plants and destroy them (McKee and Wolf 1963).[192]

Fish and benthic organisms may be affected by soluble substances extracted from the oils or by coating from emulsified oils. Water soluble compounds from crude or manufactured oils may also contain tainting substances which affect the taste of fish and waterfowl (Krishnawami and Kupchanko 1969).[189]

Toxicity tests for oily substances provide a broad range of results which do not permit rigorous safety evaluations. The variabilities are due to differences in petroleum products tested, non-uniform testing procedures, and species differences. Most of the research on the effects of oils on aquatic life has used pure compounds which exist only in low percentages in many petroleum products or crude oils. Table III-8 illustrates the range of reported toxicities. For halo-, nitro-, or thio-derivatives, the expected toxicity would be greater.

Because of the basic difficulties in evaluating the toxicity, especially of the emulsified oils, and because there is some evidence that oils may persist and have subtle chronic effects (Blumer 1971),[176] the maximum allowable concentration of emulsified oils should be determined on an individual basis and kept below 0.05 of the 96-hour LC50 for sensitive species.

TABLE III-8—Toxicity Ranges

Chemical	ppm. conc.	Effect	Species	Investigator
Aniline	370	none	Daphnia magna	Anderson 1944[17]
Benzene	31	96 hr LC50	Pimephales promelas	Pickering & Henderson 1966[181]
	22	96 hr LC50	Lepomis macrochirus	" "
	32	96 hr LC50	Carassius auratus	" "
Cresol	10	96 hr LC50	Lepomis macrochirus	Cairns & Scheier 1959[176]
Cyclohexane	30	96 hr LC50	Pimephales promelas	Pickering & Henderson 1966[181]
	31	96 hr LC50	Lepomis macrochirus	" "
	33	"	Carassius auratus	" "
	48	"	Lebistes reticulatus	" "
Ethylbenzene	48	"	Pimephales promelas	" "
	29	"	Lepomis macrochirus	" "
	73	"	Carassius auratus	" "
	78	"	Lebistes reticulatus	" "
Heptane	4924	48 hr LC50	Gambusia affinis	Wallen et al. 1957[196]
Isoprene	75	96 hr LC50	Pimephales promelas	Pickering & Henderson 1966[181]
	39	"	Lepomis macrochirus	" "
	180	"	Carassius auratus	" "
	140	"	Lebistes reticulatus	" "
Naphthenic acid	5.6	"	Lepomis macrochirus	Cairns & Scheier 1958[177]
	5.6–7.5	"	Physa heterostropha	" "
Naphthalene	165	48 hr LC50	Gambusia affinis	Wallen et al. 1957[196]
Toluene	1200	"	"	" "
	44	96 hr LC50	Pimephales promelas	Pickering & Henderson 1966[181]
	24	"	Lepomis macrochirus	" "
	62	"	Carassius auratus	" "
	65	"	Lebistes reticulatus	" "
Gasoline	91	48 hr LC50	Alosa sapidissima	Tagatz 1961[193]
	40	96 hr LC50	Salmo gairdneri	Meinck et al. 1956[180]
Cutting oil #2	14,500	96 hr LC50	Salmo gairdneri	Turnbull et al 1954[194]
Diesel fuel	167	48 hr LC50	Alosa sapidissima	Tagatz 1961[193]
Bunker oil	2417	"	Alosa sapidissima	" "
Bunker C oil	1700	100 hr LC50	Salmo salar	Sprague and Carson manuscript 1970[192]

SEDIMENTED OIL

Ludzack et al. (1957)[190] found that the sediment in the Ottawa River in Ohio downstream from a refinery consisted of up to 17.8 per cent oil. Hunt (1957)[187] and Hartung and Klingler (1968)[185] reported on the occurrence of sedimented oil in the Detroit River. North et al. (1965)[184] found sedimented oils after an oil pollution incident in marine coves in Baja California. Forbes and Richardson (1913)[181] reported 2.5 per cent oils in the bottom deposits of the Illinois River. McCauley (1964)[192] reported finding oily bottom deposits after oil pollution near Boston. Thus, while the reports may be scattered, the evidence is clear that the existence of sedimented oils in association with oil pollution is widespread.

There is an increasing body of evidence indicating that aliphatic hydrocarbons are synthesized by aquatic organisms and find their way into sediments in areas which have little or no history of oil pollution (Han et al. 1968,[183] Avigan and Blumer 1968[174]). Hydrocarbons have been reported in the recent sediments of lakes in Minnesota (Swain 1956)[212] and the Gulf of Mexico (Stevens et al. 1956).[214]

Areas which contain oily sediments usually have an impoverished benthic fauna; it is not clear to what extent oil contributes to this, because of the presence of other pollutants (Hunt 1962).[188] However, there are recurring reports of a probable relationship between sedimented oils and altered benthic communities. Sedimented oils may act as concentrators for chlorinated hydrocarbon pesticides (Hartung and Klingler 1970),[186] but the biological implications indicate that additional study is required.

Because of the differences in toxicities of sedimented oils and because of limited knowledge on quantities which are harmful to aquatic life, it is suggested that the concentration of hexane extractable substances (exclusive of elemental sulfur) in air-dried sediments not be permitted to increase above 1,000 mg/kg on a dry weight basis.

Recommendations

Aquatic life and wildlife should be protected where:

- **there is no visible oil on the surface;**
- **emulsified oils do not exceed 0.05 of the 96-hour LC50;**
- **concentration of hexane extractable substances (exclusive of elemental sulfur) in air-dried sediments does not increase above 1,000 mg/kg on a dry weight basis.**

TAINTING SUBSTANCES

Discharges from municipal wastewater treatment plants, a variety of industrial wastes and organic compounds, as well as biological organisms, can impart objectionable taste, odor, or color to the flesh of fish and other edible aquatic organisms. Such tainting can occur in waters with concentrations of the offending material lower than those recognized as being harmful to an animal (Tables III–9 and III–10).

BIOLOGICAL CAUSES OF TAINTING

Thaysen (1935)[231] and Thaysen and Pentelow (1936)[232] demonstrated that a muddy or earthy taste can be imparted to the flesh of trout by material produced by an odiferous species of *Actinomyces*. Lopinot (1962)[224] reported a serious fish and municipal water supply tainting problem on the Mississippi River in Illinois during a period when actinomycetes, *Oscillatoria*, *Scenedesmus*, and *Actinastrum* were abundant. *Oscillatoria princeps* and *O. agardhi* in plankton of a German lake were reported by Cornelius and Bandt (1933)[212] as causing off-flavor in lake fish. Aschner et al. (1967)[210] concluded that the benthic alga, *O. tenuis*, in rearing ponds in Israel was responsible for imparting such a bad flavor to carp (*Cyprinus carpio*) that the fish were unacceptable on the market. Henley's (1970)[221] investigation of odorous metabolites of Cyanophyta showed that *Anabaena circinalis* releases geosmin and indicated that this material was responsible for the musty or earthy odor often characteristic of water from reservoirs with heavy algal growths in summer and fall.

Oysters occasionally exhibit green coloration of the gills due to absorption of the blue-green pigment of the diatom, *Navicula*, (Ranson 1927).[228]

TAINTING CAUSED BY CHEMICALS

Phenolic compounds are often associated with both water and fish tainting problems (Table III–9). However, Albersmeyer (1957)[205] and Albersmeyer and Erichsen (1959)[206] found that, after being dephenolated, both a carbolated oil and a light oil still imparted a taste to fish more pronounced than that produced by similar exposures to naphthalene and methylnaphthalene (phenolated compounds). They concluded that other hydrocarbons in the oils were more responsible for imparting off-flavor than the phenolic materials in the two naphthalenes tested.

Refineries (Fetterolf 1962),[215] oily wastes (Zillich 1969),[236] and crude oil (Galtsoff et al. 1935)[218] have been associated with off-flavor problems of fish and shellfish in both freshwater and marine situations (Westman and Hoff 1963).[234] Krishnawami and Kupchanko (1969)[223] demonstrated that rainbow trout (*Salmo gairdneri*) adsorbed enough compounds from a stream polluted with oil slicks and oil refinery effluents to exhibit a definite oily taste and flavor. In waters receiving black liquor from kraft pulp mills, the gills and mantles of oysters developed a gray color (Galtsoff et al. 1947).[219] The authors also found this condition in oysters grown in waters receiving domestic sewage. Newton (1967)[227] confined trout in live-cages and correlated inten-

TABLE III–9—Wastewaters Found to have Lowered the Palatability of Fish Flesh

Wastewater source	Concentration in water affecting palatability of fish	Species	Reference
2,4-D mfg. plant	50–100 mg/l	Trout	Shumway 1966[229]
Coal—coking	0.02–0.1 mg/l	Freshwater fish	Bandt 1955[211]
Coal—tar	0.1 mg/l	Freshwater fish	Bandt 1955[211]
Kraft process (unbleached)	1–2½% by vol.	Salmon	Shumway and Chadwick 1971[230]
Kraft process (bleached)	9–17% by vol.	Salmon	Shumway and Palensky, unpublished data[239]
Kraft and neutral sulfite process	......	Trout	Newton 1967[227]
Municipal dump runoff	......	Channel catfish (Ictalurus punctatus)	Thomas and Hicks 1971[233]
Municipal untreated sewage (2 locations)	......	Channel catfish	Thomas and Hicks 1971[233]
Municipal wastewater treatment plants (4 locations)	......	Channel catfish	Thomas and Hicks 1971[233]
Municipal wastewater treatment plant (Primary)	11–19% by vol.	Freshwater fish	Shumway and Palensky, unpublished data[239]
Municipal wastewater treatment plant (Secondary)	20–26% by vol.	Freshwater fish	Shumway and Palensky, unpublished data[239]
Oily wastes	......	Trout	Zillich 1969[236]
Refinery	......	Trout	Fetterolf 1962[215]
Sewage containing phenols	0.1 mg/l	Freshwater fish	Bandt 1955[211]
Slaughterhouses (2 locations)	......	Channel catfish	Thomas and Hicks 1971[233]

TABLE III-10—*Concentrations of Chemical Compounds in Water That Can Cause Tainting of the Flesh of Fish and Other Aquatic Organisms*

Chemical	Estimated threshold level in water (mg/l)	Reference*
acetophenone	0.5	d
acrylonitrile	18	f
cresol	0.07	g
m-cresol	0.2	g
o-cresol	0.4	g
p-cresol	0.12	g
cresylic acid (meta para)	0.2	d
N-butylmercaptan	0.06	g
o-sec. butylphenol	0.3	d
p-tert. butylphenol	0.03	d
o-chlorophenol	0.0001 to 0.015	b, d, e
p-chlorophenol	0.01 to 0.05	d, g, e
2,3-dichlorophenol	0.084	g
2,4-dichlorophenol	0.001 to 0.014	d, f, g
2,5-dichlorophenol	0.023	g
2,6-dichlorophenol	0.035	g
2-methyl, 4-chlorophenol	0.075	g
2-methyl, 6-chlorophenol	0.003	g
o-phenylphenol	1	d
2,4,6-trichlorophenol	0.002 to 0.05	g
phenol	1 to 10	d, e
phenols in polluted river	0.02 to 0.15	a
diphenyl oxide	0.05	d
β, β-dichlorodiethyl ether	0.09 to 1.0	d, g
o-dichlorobenzene	0.25	d
ethylbenzene	<0.25	d
ethanethiol	0.24	g
ethylacrylate	0.6	g
formaldehyde	95	g
kerosene	0.1	d
kerosene plus kaolin	1	i
isopropylbenzene	<0.25	d
naphtha	0.1	d
naphthalene	1	d
naphthol	0.5	d
2-naphthol	0.3	g
dimethylamine	7	g
α-methylstyrene	0.25	d
oil, emulsifiable	>15	d
pyridine	5 to 28	d, g
pyrocatechol	0.8 to 5	d, g
pyrogallol	20 to 30	e
quinoline	0.5 to 1	e
p-quinone	0.5	e
styrene	0.25	d
toluene	0.25	d
outboard motor fuel, as exhaust	2.6 gal/acre-foot	c, h
guaiacol	0.082	g

* Reference key:
- a Bandt 1955[illegible]
- b Boetius 1954[illegible]
- c English et al. 1963[illegible]
- d Fetterolf 1964[illegible] published the results of A. W. Winston, Jr. of the Dow Chemical Company. The data are also available in an undated mimeographed release of the company
- e Schulze 1961[illegible]
- f Shumway 1966[illegible]
- g Shumway, D. L. and J. R. Palensky,[illegible] unpublished data (1971).
- h Surber, et al. 1965[illegible]
- i Westman and Hoff 1963[illegible]

sity of off-flavor with proximity to the discharge of a paper mill using both the neutral sulfite and kraft processes.

Shellfish have the ability to concentrate and store metals at levels greater than the concentrations in the water (see Section I, pp. 36–37, and Section IV, p. 240). Oyster flesh can become green-colored from copper accumulation. The copper content of normal-colored oyster flesh from uncontaminated areas varied from 0.170 to 0.214 mg copper per oyster, or from 8.21 to 13.77 mg per 100 grams dry weight (Galtsoff and Whipple 1931,[220] Galtsoff 1964[217]). Oysters growing in adjacent areas slightly contaminated with copper salts had green-colored flesh and contained from 1.27 to 2.46 mg copper per oyster, or from 121 to 271 mg per 100 grams dry weight.

If an effluent containing a variety of components is associated with a tainting problem, identification of the taint-producing component or components is necessary for efficient isolation and removal in waste treatment. For example, Shumway (1966)[223] exposed salmon to various concentrations of wastes and waste components discharged from a plant producing pesticides. Although concentrations of the combined wastes at about 50 to 100 mg/l were found to impart objectionable flavor to test fish, one of the major components of the plant waste, 2,4-dichlorophenol, was found capable of impairing flavor at exposure levels of about 1 to 3 μg/l.

A preliminary laboratory study (English et al. 1963)[214] showed that outboard motor exhaust damages the quality of water in several ways, the most noticeable of which are unpleasant taste and odor in the water and off-flavoring of fish flesh. A later field study (Surber et al. 1965)[235] determined the threshold level of tainting of fish in pond and lake waters to be about 2.6 gal/acre-foot of fuel as exhaust, accumulating over a 2-month period. The gasoline used was regular grade, and the lubricating oil (½ pint/gal) was a popular brand of packaged outboard motor oil.

UPTAKE AND LOSS OF FLAVOR-IMPAIRING MATERIALS

Experiments involving method and rates of uptake and loss of flavor-impairing materials by aquatic organisms have been reported by few investigators. From data available it is obvious that rates are highly variable. Thaysen and Pentelow (1936)[232] exposed trout to extract from odoriferous *Actinomyces*. They showed that fish exposed to 10 ppm of extract acquired an off-flavor in one hour. The exposed fish were also removed and held in uncontaminated water for periods up to five days. The level of tainting, which showed no diminution after 27 hours, became less marked after 2 to 3 days, and no tainting could be detected after 5 days in fresh water.

Shumway and Palensky (*unpublished data*)[229] exposed trout to three separate concentrations of each of the following chemicals, *o*-cresol, 2,4-dichlorophenol, pyridine, and *n*-butylmercaptan, for periods up to 168 hours. With all four chemicals, maximum off-flavor generally occurred in 33.5 hours or less. In a few exceptions, a gradual increase in off-flavor appeared to occur with increasing time up to 168 hours, although the magnitude of increase in off-flavor with time was minor in nature. In tests with *o*-chlorophenol, Boetius (1954)[313] reported that eels required up to 11 days exposure before flavor impairment was detected. The time required to impair flavor was found to be related to the exposure concentration, with low concentrations requiring longer exposure periods.

Shumway (1966)[329] found that the flesh of salmon exposed experimentally to industrial wastes containing mainly phenols acquired maximum off-flavor in 35 hours or less, with much of the tainting occurring within the first 6 hours. After the salmon were transferred to uncontaminated water, most of the acquired off-flavor was lost within 20 hours, although some off-flavor remained up to 72 hours.

In other tests, Shumway and Palensky (*unpublished data*)[330] observed flavor impairment in trout after 24-hour exposure to 2,4-dichlorophenol. After only 33.5 hours in uncontaminated water, the flavor of the trout had returned to the preexposure level, with most of the reduction in off-flavor occurring within 6.5 hours.

Korschgen et al. (1970)[322] transferred carp (*Cyprinus carpio*) to uncontaminated ponds from two sites, one of which received effluents from a major municipality and one of which received little or no effluent. Retention up to 18 days in the holding ponds failed to improve the flavor of the carp from the contaminated site. These authors also reported that channel catfish (*Ictalurus punctatus*) transferred from the Ohio River to control water lost about half of their off-flavor in 7 days and nearly all of it in 21 days.

IDENTIFICATION OF CAUSES OF OFF-FLAVORED ORGANISMS

Determination that a tainting problem exists, or identification of a taint-causing material, involves field or laboratory exposure periods and organoleptic tests. When properly conducted, these tests are reliable but time-consuming. Wright (1966)[344] reported on the use of gas chromatography in conjunction with organoleptic tests. The chromatographic scans were compared with scans of industrial process waste streams to identify the taint-producing wastes. Gas chromatographic techniques are employed routinely in food technology laboratories investigating flavor and odor properties (Rhoades and Millar 1965).[326]

EXPOSURE AND ORGANOLEPTIC TESTS

Field exposure tests (bioassays) are used to determine the existence or the magnitude of a tainting problem in a water body. Fish or other edible aquatic life are held for a period of time in cages at selected locations in and around a suspected problem area or waste discharge and eventually evaluated for flavor. Laboratory bioassays are normally utilized to determine the tainting potential of wastes, waste components, or specific chemicals. Although either static or continuous-flow bioassays can be used in laboratory tests, continuous-flow systems are considered far superior to static tests. Exposure bioassays are followed by the organoleptic evaluation of the flesh of the test organisms.

In their studies of tainted organisms, investigators have used a number of different bioassay and flavor-evaluation procedures, some of which have produced poorly defined results. The following guidelines are based primarily on the successful procedures of Shumway and Newton (*personal communications*).[329]

Test Fish

The flesh of the fish to be exposed should be mild and consistent in flavor. For convenience in holding and taste testing, fish weighing between 200 and 400 grams are desirable, although smaller or larger fish are acceptable. Largemouth bass (*Micropterus salmoides*), yellow perch (*Perca flavescens*), channel catfish, bluegill (*Lepomis macrochirus*), trout, salmon flatfishes (Pleuronectiformes), and others have proven to be acceptable test fish.

Exposure Period

In general, test fish should be exposed for a period not less than 48 hours. Shorter or longer exposures will be advisable in some situations, although possible stress, disease, and mortality resulting from longer retention of test fish and maintenance of holding facilities may negate advantages of long exposure.

Exposure Conditions

The following conditions are desirable in laboratory bioassays:

Dissolved oxygen	near saturation
Temperature	10–15 C for salmonids, and 20–25 C for warmwater fish
pH	6.0–8.0, or pH of receiving water
Light	intensity held at a low level
Water	uncontaminated, or quality of the receiving water; never distilled water

Preparation of Test Fish and Evaluation

Exposed fish and control fish, either fresh or fresh-frozen and subsequently thawed, are individually double-wrapped in aluminum foil, placed in an oven and cooked at about 375 F for 15 to 30 minutes, as size requires. Large fish may be portioned for cooking. No seasoning of any kind is added. Portions of the cooked fish may be placed in small coded cups and served warm to the judges for flavor evaluation. A known "reference" may be provided to aid judges in making comparisons. A minimum of ten experienced judges, each seated in an isolation booth or similar area, smell, taste, and score each sample. This method offers tighter control of variables and conforms more to off-flavor evaluations conducted in food laboratories than the more informal procedure below.

An alternative method is to place the cooked fish, still partially wrapped in foil to preserve the heat and flavor, on a large table. The judges start concurrently and work their way around the table, recording aroma and flavor. If a judge tastes more than six samples during a test, a lessening of organoleptic acuity may occur.

When investigating the potential of a substance to produce taint, a word-evaluation scale for intensity of off-flavor ranging from no off-flavor to extreme off-flavor, has proven successful with trained, experienced judges. Numerical values from 0 to 6 are applied to the word scale for derivation of off-flavor indices and statistical evaluation.

When using the above method, less experienced judges tend to over-react to slight off-flavor. For this reason, in less formal tests evaluating the effect of a substance on the palatability of the organism, an hedonic scale accompanied by word-judgments describing palatability is appropriate, i.e., 0—excellent, 1—very good, 2—good, 3—fair, 4—just acceptable, 5—not quite acceptable, 6—very poor, inedible, and 7—extremely poor, repulsive. Scores of the judges on each sample are averaged to determine final numerical or word-judgment values.

To determine whether there are acceptability differences between controls and test organisms, a triangle test may be used in which two samples are alike and one is different. Judges are asked to select the like samples, to indicate the degree of difference, and to rate both the like and the odd samples on a preference scale.

STATISTICAL EVALUATION

The triangle test is particularly well adapted to statistical analysis, but the organoleptic testing necessary is more extensive than when hedonic scales are used.

Application of the two-way analyses of variance to hedonic-scale data is an acceptable test, but professional assistance with statistical procedures is desirable. Reliance on the word-judgment system is sufficient for general information purposes.

Recommendations

- **To prevent tainting of fish and other edible aquatic organisms, it is recommended that substances which cause tainting should not be present in water in concentrations that lower the acceptability of such organisms as determined by exposure bioassay and organoleptic tests.**
- **Values in Tables III–9 and III–10 are recommended as guidelines in determining what concentrations of wastes and substances in water may cause tainting of the flesh of fish or other aquatic organisms.**

HEAT AND TEMPERATURE

Living organisms do not respond to the quantity of heat but to degrees of temperature or to temperature changes caused by transfer of heat. The importance of temperature to acquatic organisms is well known, and the composition of aquatic communities depends largely on the temperature characteristics of their environment. Organisms have upper and lower thermal tolerance limits, optimum temperatures for growth, preferred temperatures in thermal gradients, and temperature limitations for migration, spawning, and egg incubation. Temperature also affects the physical environment of the aquatic medium, (e.g., viscosity, degree of ice cover, and oxygen capacity. Therefore, the composition of aquatic communities depends largely on temperature characteristics of the environment. In recent years there has been an accelerated demand for cooling waters for power stations that release large quantities of heat, causing, or threatening to cause, either a warming of rivers, lakes, and coastal waters, or a rapid cooling when the artificial sources of heat are abruptly terminated. For these reasons, the environmental consequences of temperature changes must be considered in assessments of water quality requirements of aquatic organisms.

The "natural" temperatures of surface waters of the United States vary from 0 C to over 40 C as a function of latitude, altitude, season, time of day, duration of flow, depth, and many other variables. The agents that affect the natural temperature are so numerous that it is unlikely that two bodies of water, even in the same latitude, would have exactly the same thermal characteristics. Moreover, a single aquatic habitat typically does not have uniform or consistent thermal characteristics. Since all aquatic organisms (with the exception of aquatic mammals and a few large, fast-swimming fish) have body temperatures that conform to the water temperature, these natural variations create conditions that are optimum at times, but are generally above or below optima for particular physiological, behavioral, and competitive functions of the species present.

Because significant temperature changes may affect the composition of an aquatic or wildlife community, an induced change in the thermal characteristics of an ecosystem may be detrimental. On the other hand, altered thermal characteristics may be beneficial, as evidenced in most fish hatchery practices and at other aquacultural facilities. (See the discussion of Aquaculture in Section IV.)

The general difficulty in developing suitable criteria for temperature (which would limit the addition of heat) lies in determining the deviation from "natural" temperature a particular body of water can experience without suffering adverse effects on its biota. Whatever requirements are suggested, a "natural" seasonal cycle must be retained, annual spring and fall changes in temperature must be gradual, and large unnatural day-to-day fluctuations should be avoided. In view of the many variables, it seems obvious that no single temperature requirement can be applied uniformly to continental or large regional areas; the requirements must be closely related to each body of water and to its particular community of organisms, especially the important species found in it. These should include invertebrates, plankton, or other plant and animal life that may be of importance to food chains or otherwise interact with species of direct interest to man. Since thermal requirements of various species differ, the social choice of the species to be protected allows for different "levels of protection" among water bodies as suggested by Doudoroff and Shumway (1970)[272] for dissolved oxygen criteria. (See Dissolved Oxygen, p. 131.) Although such decisions clearly transcend the scientific judgments needed in establishing thermal criteria for protecting selected species, biologists can aid in making them. Some measures useful in assigning levels of importance to species are: (1) high yield to commercial or sport fisheries, (2) large biomass in the existing ecosystem (if desirable), (3) important links in food chains of other species judged important for other reasons, and (4) "endangered" or unique status. If it is desirable to attempt strict preservation of an existing ecosystem, the most sensitive species or life stage may dictate the criteria selected.

Criteria for making recommendations for water temperature to protect desirable aquatic life cannot be simply a maximum allowed change from "natural temperatures." This is principally because a change of even one degree from an ambient temperature has varying significance for an organism, depending upon where the ambient level lies within the tolerance range. In addition, historic temperature records or, alternatively, the existing ambient temperature prior to any thermal alterations by man are not always reliable indicators of desirable conditions for aquatic populations. Multiple developments of water resources also change water temperatures both upward (e.g., upstream power plants or shallow reservoirs) and downward (e.g., deepwater releases from large reservoirs), so that "ambient" and "natural" are exceedingly difficult to define at a given point over periods of several years.

Criteria for temperature should consider both the multiple thermal requirements of aquatic species and requirements for balanced communities. The number of distance requirements and the necessary values for each require periodic reexamination as knowledge of thermal effects on aquatic species and communities increases. Currently definable requirements include:

- maximum sustained temperatures that are consistent with maintaining desirable levels of productivity;
- maximum levels of metabolic acclimation to warm temperatures that will permit return to ambient winter temperatures should artificial sources of heat cease;
- temperature limitations for survival of brief exposures to temperature extremes, both upper and lower;
- restricted temperature ranges for various stages of reproduction, including (for fish) gonad growth and gamete maturation, spawning migration, release of gametes, development of the embryo, commencement of independent feeding (and other activities) by juveniles; and temperatures required for metamorphosis, emergence, and other activities of lower forms;
- thermal limits for diverse compositions of species of aquatic communities, particularly where reduction in diversity creates nuisance growths of certain organisms, or where important food sources or chains are altered;
- thermal requirements of downstream aquatic life where upstream warming of a cold-water source will adversely affect downstream temperature requirements.

Thermal criteria must also be formulated with knowledge of how man alters temperatures, the hydrodynamics of the changes, and how the biota can reasonably be expected to interact with the thermal regimes produced. It is not sufficient, for example, to define only the thermal criteria for sustained production of a species in open waters, because large numbers of organisms may also be exposed to thermal changes by being pumped through the condensers and mixing zone of a power plant. Design engineers need particularly to know the biological limitations to their design options in such instances. Such considerations may reveal nonthermal impacts of cooling processes that may outweigh temperature effects, such as impingement of fish upon intake screens, mechanical or chemical damage to zooplankton in condensers, or effects of altered current patterns on bottom fauna in a discharge area. The environmental situations of aquatic organisms (e.g., where they are, when they are there, in what numbers) must also be understood. Thermal criteria for migratory species should be applied to a certain area only when the species is actually there. Although thermal effects of power stations are currently of great interest, other less dramatic causes of temperature change including deforestation, stream channelization, and impoundment of flowing water must be recognized.

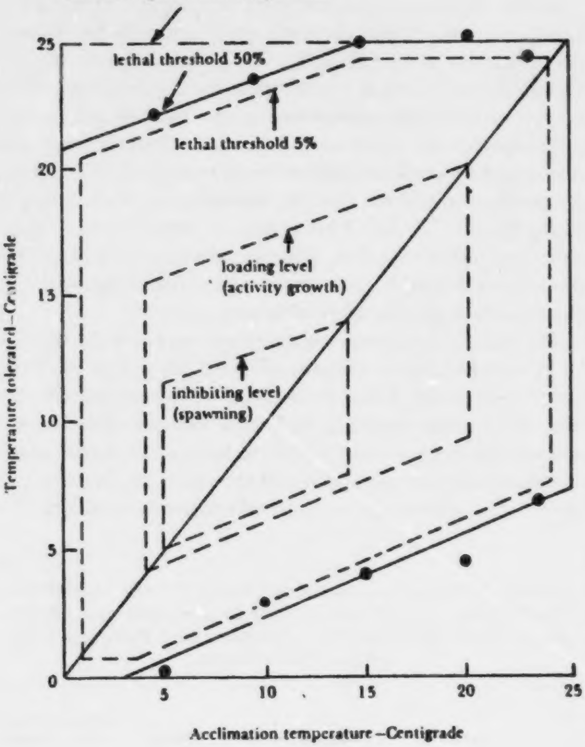
DEVELOPMENT OF CRITERIA

Thermal criteria necessary for the protection of species or communities are discussed separately below. The order of presentation of the different criteria does not imply priority for any one body of water. The descriptions define preferred methods and procedures for judging thermal requirements, and generally do not give numerical values (except in Appendix II–C). Specific values for all limitations would require a biological handbook that is far beyond the scope of this Section. The criteria may seem complex, but they represent an extensively developed framework of knowledge about biological responses. (A sample application of these criteria begins on page 166, Use of Temperature Criteria.)

TERMINOLOGY DEFINED

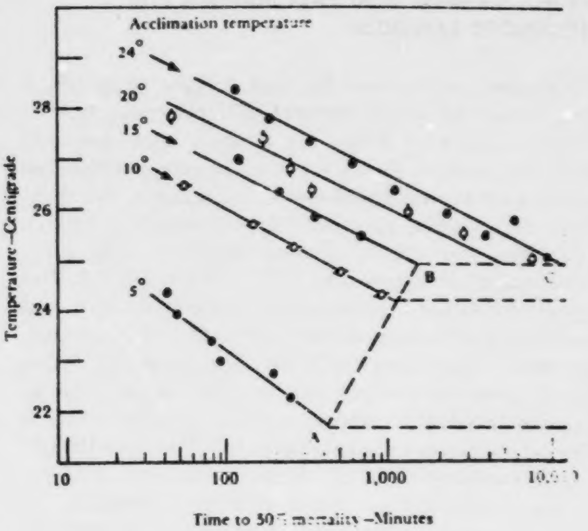
Some basic thermal responses of aquatic organisms will be referred to repeatedly and are defined and reviewed briefly here. Effects of heat on organisms and aquatic communities have been reviewed periodically (e.g., Bullock 1955,[259] Brett 1956;[253] Fry 1947,[276] 1964,[275] 1967;[279] Kinne 1970[296]). Some effects have been analyzed in the context of thermal modification by power plants (Parker and Krenkel 1969;[305] Krenkel and Parker 1969;[298] Cairns 1968;[261] Clark 1969;[263] and Coutant 1970c[269]). Bibliographic information is available from Kennedy and Mihursky (1967),[294] Raney and Menzel (1969),[313] and from annual reviews published by the Water Pollution Control Federation (Coutant 1968,[265] 1969,[266] 1970a,[267] 1971[270]).

Each species (and often each distinct life-stage of a species) has a characteristic tolerance range of temperature as a consequence of acclimations (internal biochemical adjustments) made while at previous holding temperature (Figure III–2; Brett 1956[253]). Ordinarily, the ends of this range, or the lethal thresholds, are defined by survival of 50 per cent of a sample of individuals. Lethal thresholds typically are referred to as "incipient lethal temperatures," and temperature beyond these ranges would be considered "extreme." The tolerance range is adjusted upward by acclimation to warmer water and downward to cooler water, although there is a limit to such accommodation. The low end of the range usually is at zero degrees centigrade (32 F) for species in temperate latitudes (somewhat less for saline waters), while the upper end terminates in an "ultimate incipient lethal temperature" (Fry et al. 1946[281]). This ultimate threshold temperature represents the "breaking point" between the highest temperatures to which an animal can be acclimated and the lowest of the extreme temperatures that will kill the warm-acclimated organism. Any rate of temperature change over a period of minutes to a few hours will not greatly affect the thermal tolerance limits, since acclimation to changing temperatures requires several days (Brett 1941).[251]



After Brett 1960[254]

FIGURE III-2—*Upper and lower lethal temperatures for young sockeye salmon (Oncorhynchus nerka) plotted to show the zone of tolerance. Within this zone two other zones are represented to illustrate (1) an area beyond which growth would be poor to none-at-all under the influence of the loading effect of metabolic demand, and (2) an area beyond which temperature is likely to inhibit normal reproduction.*



After Brett 1952[252]

FIGURE III-3—*Median resistance times to high temperatures among young chinook (Oncorhynchus tshawytscha) acclimated to temperatures indicated. Line A-B denotes rising lethal threshold (incipient lethal temperatures) with increasing acclimation temperature. This rise eventually ceases at the ultimate lethal threshold (ultimate upper incipient lethal temperature), line B-C.*

At the temperatures above and below the incipient lethal temperatures, survival depends not only on the temperature but also on the duration of exposure, with mortality occurring more rapidly the farther the temperature is from the threshold (Figure III-3). (See Coutant 1970a[257] and 1970b[268] for further discussion based on both field and laboratory studies.) Thus, organisms respond to extreme high and low temperatures in a manner similar to the dosage-response pattern which is common to toxicants, pharmaceuticals, and radiation (Bliss 1937).[249] Such tests seldom extend beyond one week in duration.

MAXIMUM ACCEPTABLE TEMPERATURES FOR PROLONGED EXPOSURES

Specific criteria for prolonged exposure (1 week or longer) must be defined for warm and for cold seasons. Additional criteria for gradual temperature (and life cycle) changes during reproduction and development periods are discussed on pp. 162–165.

SPRING, SUMMER, AND FALL MAXIMA FOR PROLONGED EXPOSURE

Occupancy of habitats by most aquatic organisms is often limited within the thermal tolerance range to temperatures somewhat below the ultimate upper incipient lethal temperature. This is the result of poor physiological performance at near lethal levels (e.g., growth, metabolic scope for activities, appetite, food conversion efficiency), interspecies competition, disease, predation, and other subtle ecological factors (Fry 1951;[277] Brett 1971[250]). This complex limitation is evidenced by restricted southern and altitudinal distributions of many species. On the other hand, optimum temperatures (such as those producing fastest growth rates) are not generally necessary at all times to maintain thriving populations and are often exceeded in nature during summer months (Fry 1951;[277] Cooper 1953;[264] Beyerle and Cooper 1960;[246] Kramer and Smith 1960[297]). Moderate temperature fluctuations can generally be tolerated as long as a maximum upper limit is not exceeded for long periods.

A true temperature limit for exposures long enough to reflect metabolic acclimation and optimum ecological performance must lie somewhere between the physiological optimum and the ultimate upper incipient lethal temperatures. Brett (1960)[254] suggested that a provisional long-term exposure limit be the temperature greater than optimum that allowed 75 per cent of optimum performance. His suggestion has not been tested by definitive studies.

Examination of literature on performance, metabolic rate, temperature preference, growth, natural distribution, and tolerance of several species has yielded an apparently sound theoretical basis for estimating an upper temperature limit for long term exposure and a method for doing this with a minimum of additional research. New data will provide refinement, but this method forms a useful guide for the present time. The method is based on the general observations summarized here and in Figure III–4(a, b, c).

1. Performances of organisms over a range of temperatures are available in the scientific literature for a variety of functions. Figures III–4a and b show three characteristic types of responses numbered 1 through 3, of which types 1 and 2 have coinciding optimum peaks. These optimum temperatures are characteristic for a species (or life stage).

2. Degrees of impairment from optimum levels of various performance functions are not uniform with increasing temperature above the optimum for a single species. The most sensitive function appears to be growth rate, for which a temperature of zero growth (with abundant food) can be determined for important species and life stages. Growth rate of organisms appears to be an integrator of all factors acting on an organism. Growth rate should probably be expressed as net biomass gain or net growth (McCormick et al. 1971)[302] of the population, to account for deaths.

3. The maximum temperature at which several species are consistently found in nature (Fry 1951;[277] Narver 1970)[308] lies near the average of the optimum temperature and the temperature of zero net growth.

4. Comparison of patterns in Figures III–4a and b among different species indicates that while the trends are similar, the optimum is closer to the lethal level in some species than it is in sockeye salmon. Invertebrates exhibit a pattern of temperature effects on growth rate that is very similar to that of fish (Figure III–4c).

The optimum temperature may be influenced by rate of feeding. Brett et al. (1969)[257] demonstrated a shift in optimum toward cooler temperatures for sockeye salmon when ration was restricted. In a similar experiment with channel catfish, Andrews and Stickney (1972)[243] could see no such shift. Lack of a general shift in optimum may be due to compensating changes in activity of the fish (Fry *personal observation*).[326]

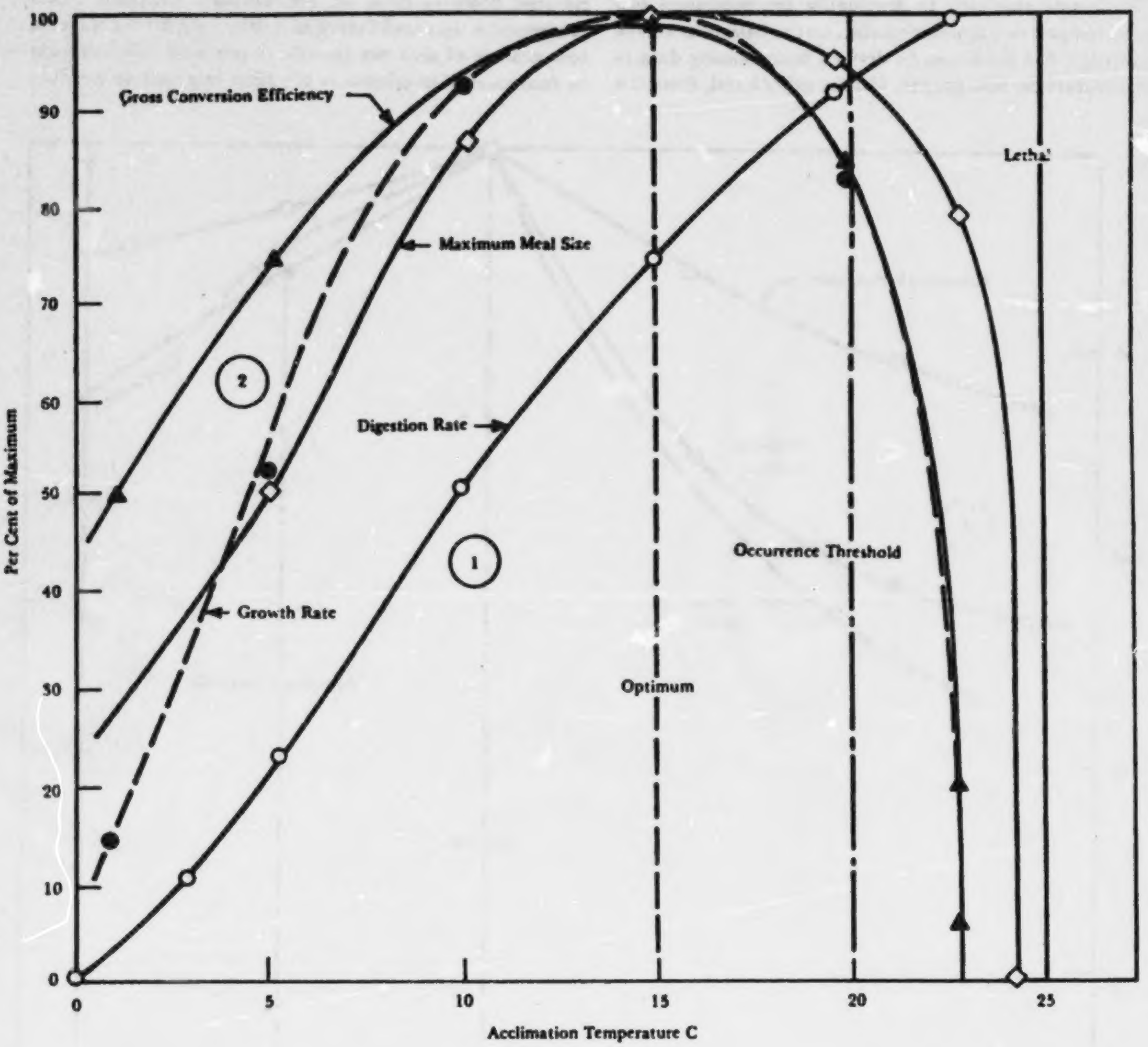
These observations suggest that an average of the optimum temperature and the temperature of zero net growth [(opt. temp. + z.n.g. temp)/2] would be a useful estimate of a limiting weekly mean temperature for resident organisms, providing the peak temperatures do not exceed values recommended for short-term exposures. Optimum growth rate would generally be reduced to no lower than 80 per cent of the maximum if the limiting temperature is as averaged above (Table III–11). This range of reduction from optimum appears acceptable, although there are no quantitative studies available that would allow the criterion to be based upon a specific level of impairment.

The criteria for maximum upper temperature must allow for seasonal changes, because different life stages of many species will have different thermal requirements for the average of their optimum and zero net growths. Thus a juvenile fish in May will be likely to have a lower maximum acceptable temperature than will the same fish in July, and this must be reflected in the thermal criteria for a waterbody.

TABLE III–11—Summary of Some Upper Limiting Temperatures in C, (for periods longer than one week) Based Upon Optimum Temperatures and Temperatures of Zero Net Growth.

Species	Optimum	Zero net growth	Reference	$\frac{\text{opt}+\text{z.n.g.}}{2}$	% of optimum
Catostomus commersoni (white sucker).....	27	29.6	*	28.3	90
Coregonus artedii (cisco or lake herring)....	16	21.2	McCormick et al. 1971[302]	18.6	82
Ictalurus punctatus (channel catfish)........	30	35.7	Strawn 1970[320]	32.8	94
....″..................................	30	35.7	Andrews and Stickney 1972[243]	32.8	88
Lepomis macrochirus (bluegill) (year II).....	22	28.6	McCormick 1971[301]	25.3	82
Micropterus salmoides (largemouth bass)....	27.5	34	Strawn 1961[319]	30.8	83
Notropis atherinoides (emerald shiner)......	27	33	*	30.5	83
Salvelinus fontinalis (brook trout)	15.4	18.8	*	17.1	89

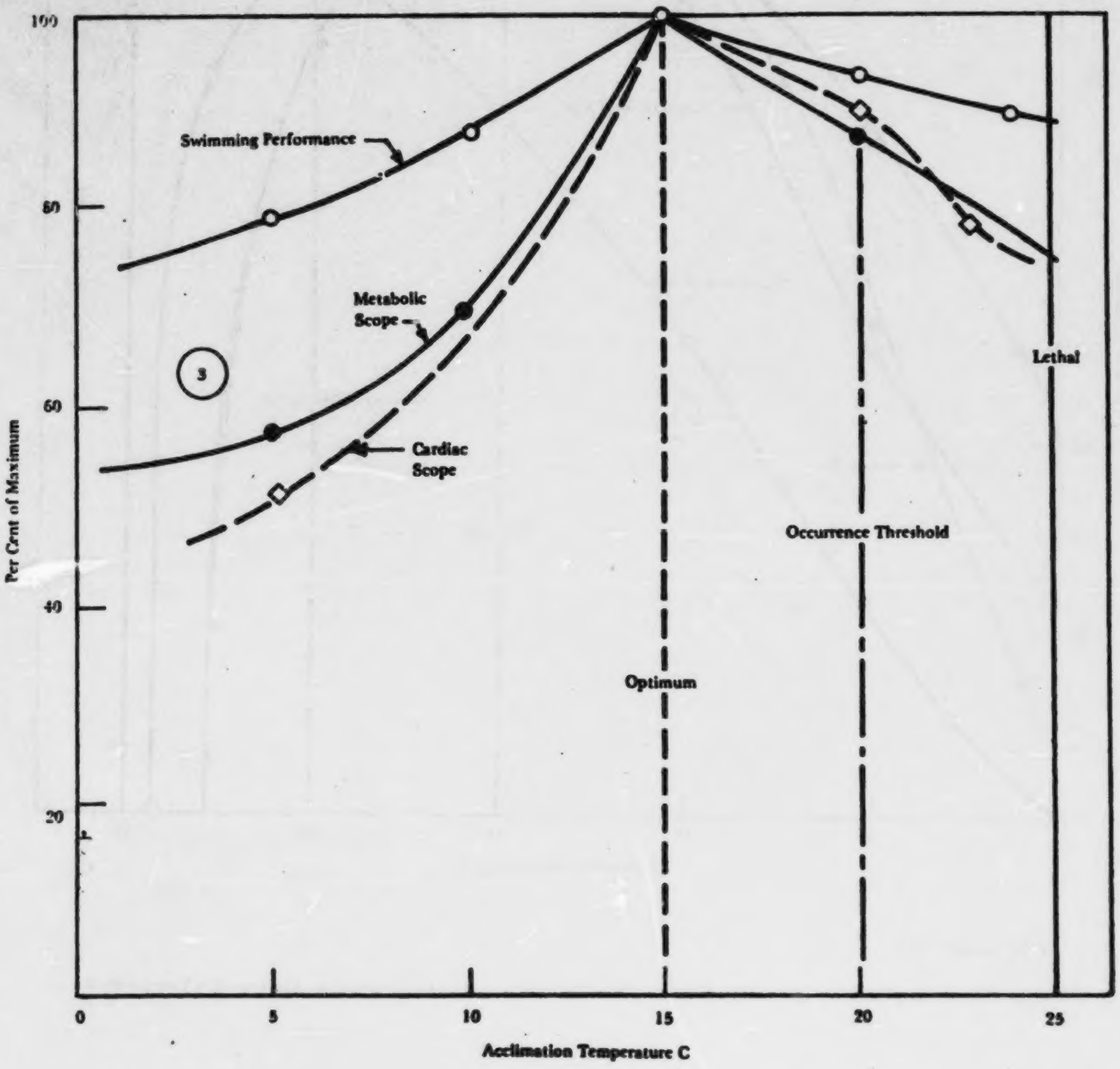
*National Water Quality Laboratory, Duluth, Minn., unpublished data.[328]



After Brett 1971[256]

FIGURE III–4a—Performance of Sockeye Salmon* (Oncorhynchus nerka) *in Relation to Acclimation Temperature

While this approach to developing the maximum sustained temperature appears justified on the basis of available knowledge, few limits can be derived from existing data in the literature on zero growth. On the other hand, there is a sizeable body of data on the ultimate incipient lethal temperature that could serve as a substitute for the data on temperature of zero net growth. A practical consideration in recommending criteria is the time required to conduct



After Brett 1971[256]

FIGURE III-4b—Performance of Sockeye Salmon* (Oncorhynchus nerka) *in Relation to Acclimation Temperature

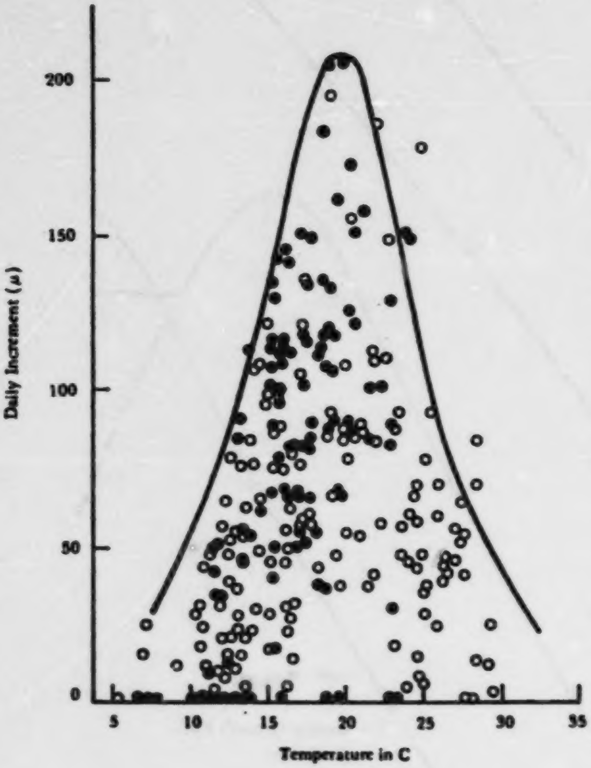
research necessary to provide missing data. Techniques for determining incipient lethal temperatures are standardized (Brett 1952)[302] whereas those for zero growth are not.

A temperature that is one-third of the range between the optimum temperature and the ultimate incipient lethal temperature that can be calculated by the formula

$$\text{optimum temp.} + \frac{\text{ultimate incipient lethal temp.} - \text{optimum temp.}}{3}$$

(*Equation I*)

yields values that are very close to (optimum temp. + z.n.g. temp.)/2. For example, the values are, respectively, 32.7 and 32.8 C for channel catfish and 30.6 and 30.8 for largemouth bass (data from Table III–8 and Appendix II). This formula offers a practical method for obtaining allowable limits, while retaining as its scientific basis the requirements of preserving adequate rates of growth. Some limits obtained from data in the literature are given in Table III–12. A hypothetical example of the effect of this limit on growth of largemouth bass is illustrated in Figure III–5.



Ansell 1968 [243]

FIGURE III–4c—M. mercenaria: *The general relationship between temperature and the rate of shell growth, based on field measurements of growth and temperature.*

●: sites in Poole Harbor, England; ○: North American sites.

Figure III–5 shows a hypothetical example of the effects of the limit on maximum weekly average temperature on growth rates of juvenile largemouth bass. Growth data as a function of temperature are from Strawn 1961[313]; the ambient temperature is an averaged curve for Lake Norman, N. C., adapted from data supplied by Duke Power Company. A general temperature elevation of 10 F is used to provide an extreme example. Incremental growth rates (mm/wk) are plotted on the main figure, while annual accumulated growth is plotted in the inset. Simplifying assumptions were that growth rates and the relationship of growth rate to temperature were constant throughout the year, and that there would be sufficient food to sustain maximum attainable growth rates at all times.

The criterion for a specific location would be determined by the most sensitive life stage of an important species likely to be present in that location at that time. Since many fishes have restricted habitats (e.g., specific depth zones) at many life stages, the thermal criterion must be applied to the proper zone. There is field evidence that fish avoid localized areas of unfavorably warm water. This has been demonstrated both in lakes where coldwater fish normally evacuate warm shallows in summer (Smith 1964)[318] and at power station mixing zones (Gammon 1970;[282] Merriman et al. 1965).[304] In most large bodies of water there are both vertical and horizontal thermal gradients that mobile organisms can follow to avoid unfavorable high (or low) temperatures.

The summer maxima need not, therefore, apply to mixing zones that occupy a small percentage of the suitable habitat or necessarily to all zones where organisms have free egress to cooler water. The maxima must apply, however, to restricted local habitats, such as lake hypolimnia or thermoclines, that provide important summer sanctuary areas for cold-water species. Any avoidance of a warm area not part of the normal seasonal habitat of the species will mean that less area of the water body is available to support the population and that production may be reduced. Such reduction should not interfere with biological communities or populations of important species to a degree that is damaging to the ecosystem or other beneficial uses. Nonmobile organisms that must remain in the warm zone will probably be the limiting organisms for that location. Any recommendation for upper limiting temperatures must be applied carefully with understanding of the population dynamics of the species in question in order to establish both local and regional requirements.

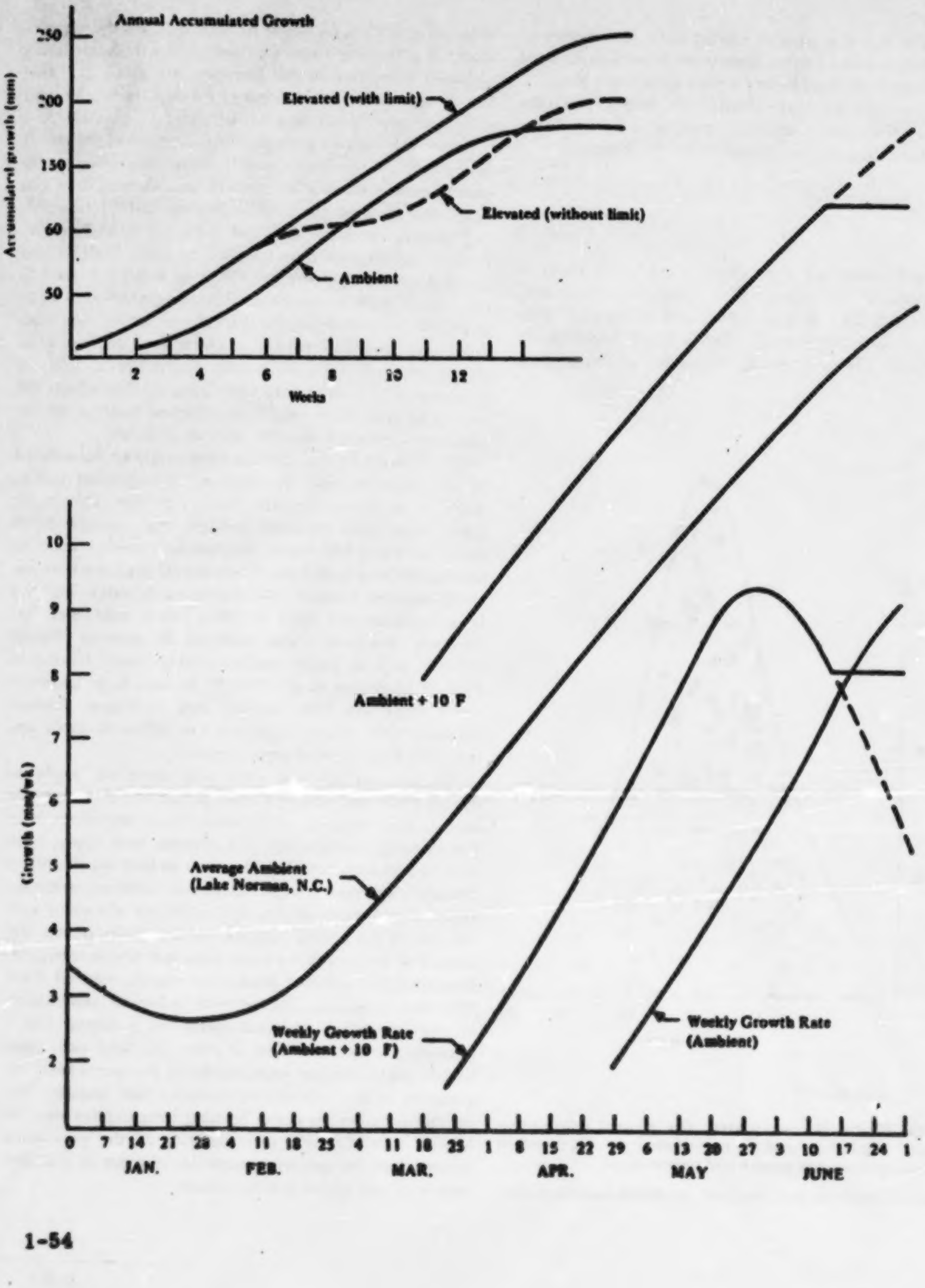

Annual Accumulated Growth
Accumulated growth (mm)
250
200
150
60
50
Elevated (with limit)
Elevated (without limit)
Ambient
2
4
6
8
10
12
Weeks
Growth (mm/wk)
10
9
8
7
6
5
4
3
2
Ambient + 10 F
Average Ambient
(Lake Norman, N.C.)
Weekly Growth Rate
(Ambient + 10 F)
Weekly Growth Rate
(Ambient)
0
7
14
21
28
4
11
18
25
4
11
18
25
1
8
15
22
29
6
13
20
27
3
10
17
24
1
JAN.
FEB.
MAR.
APR.
MAY
JUNE

FIGURE III-6—A hypothetical example of the effects of the limit on maximum weekly average temperature on growth rates of juvenile largemouth bass. Growth data as a function of temperature are from Strawn 1961; the ambient temperature is an averaged curve for Lake Norman, N.C., adapted from data supplied by Duke Power Company. A general temperature elevation of 10 F is used to provide an extreme example. Incremental growth rates (mm/wk) are plotted on the main figure, while annual accumulated growth is plotted in the inset. Simplifying assumptions were that growth rates and the relationship of growth rate to temperature were constant throughout the year, and that there would be sufficient food to sustain maximum attainable growth rates at all times.

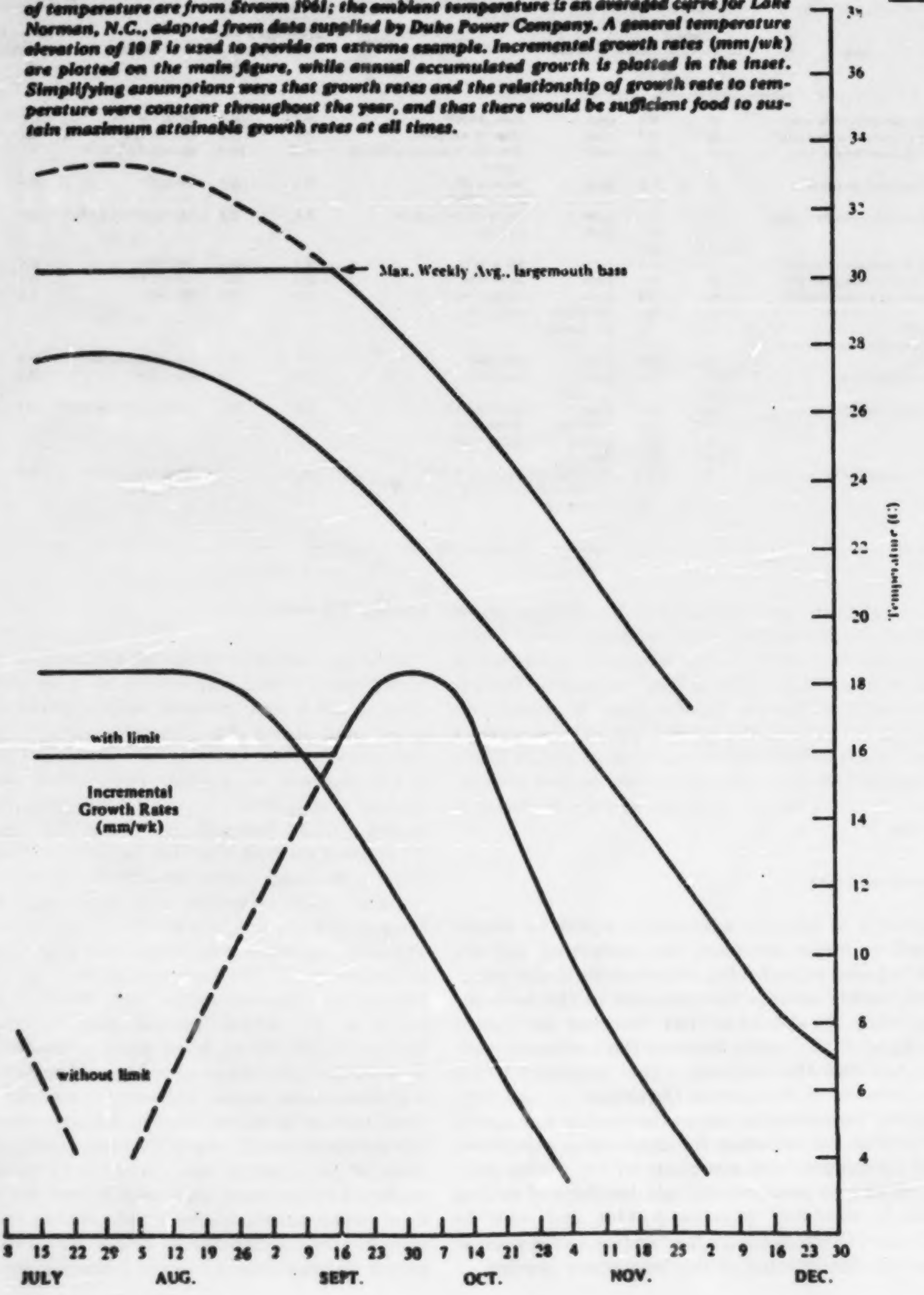


TABLE III-12—Summary of Some Upper Limiting Temperatures for Prolonged Exposures of Fishes Based on Optimum Temperatures and Ultimate Upper Incipient Lethal Temperatures (Equation 1).

Species	Optimum C	Optimum F	Function	Reference	Ultimate upper incipient lethal temperature C	Ultimate upper incipient lethal temperature F	Reference	Maximum weekly average temperature (Eq 1) C	Maximum weekly average temperature (Eq 1) F
Catostomus commersoni (white sucker)	27	80.6	growth	unpubl., NWQL	29.3	84.7	Hart 1947	27.8	82
Coregonus artedii (Cisco or lake herring)	16	60.8	growth	McCormick et al. 1971	25.7	78.3	Edsall and Colby 1970	19.2	66.6
Ictalurus punctatus (channel catfish)	30	86	growth	Strawn 1970; Andrews and Stickney 1971	38.0	100.4	Allen and Strawn 1968	32.7	90.9
Lepomis macrochirus (bluegill) (yr II)	22	71.6	growth	McComish 1971 Anderson 1959	33.8	92.8	Hart 1952	25.9	78.6
Micropterus dolomieui (smallmouth bass)	26.3	79	growth	Horning and Pearson 1972	35.0	95.0	Horning and Pearson 1972	29.9	85.8
	28.3	83	growth	Peek 1965					
	ave 27.3	81.1							
Micropterus salmoides (largemouth bass)(fry)	27.5	81.5	growth	Strawn 1961	36.4	97.5	Hart 1952	30.5	86.7
Notropis atherinoides (emerald shiner)	27	80.6	growth	unpubl., NWQL	30.7	87.3	Hart 1952	28.2	82.8
Oncorhynchus nerka (sockeye salmon)	15.0	59.0	growth	Brett et al. 1969	25.0	77.0	Brett 1952	18.3	64.9
	15.0	59.0	other functions	Brett 1971					
(juveniles)	15.0		max. swimming						
Pseudopleuronectes Americanus (winter flounder)	18.0	64.4	growth	Brett 1970	29.1	84.4	Hoff and Westman 1966	21.8	71.2
Salmo trutta (brown trout)	8 to 17	54.5	growth	Brett 1938	23.5	74.3	Bishai 1960	16.2	61.2
	ave 12.5								
Salvelinus fontinalis (brook trout)	15.4	59.7	growth	unpubl, NWQL	25.3	77.5	Fry, Hart and Walker, 1946	18.2	64.8
	13.0	55.4	growth	Baldwin 1957					
	15	59	metabolic	Graham 1949					
	ave 14.5	58.1	scope						
Salvelinus namaycush (lake trout)	16	60.8	scope for activity (2 [illegible])	Gibson and Fry 1954	23.5		Gibson and Fry 1954	18.8	65.8
	17	62.6	swimming speed						
	ave 16.5	61.7							

Heat added to upper reaches of some cold rivers can be retained throughout the river's remaining length (Jaske and Synoground 1970).[292] This factor adds to the natural trend of warming at distances from headwaters. Thermal additions in headwaters, therefore, may contribute substantially to reduction of cold-water species in downstream areas (Mount 1970).[305] Upstream thermal additions should be evaluated for their effects on summer maxima at downstream locations, as well as in the immediate vicinity of the heat source.

Recommendation

Growth of aquatic organisms would be maintained at levels necessary for sustaining actively growing and reproducing populations if the maximum weekly average temperature in the zone inhabited by the species at that time does not exceed one-third of the range between the optimum temperature and the ultimate upper incipient lethal temperature of the species (Equation 1, page 157), and the temperatures above the weekly average do not exceed the criterion for short-term exposures. This maximum need not apply to acceptable mixing zones (see proportional relationships of mixing zones to receiving systems, p. 114), and must be applied with adequate understanding of the normal seasonal distribution of the important species.

WINTER MAXIMA

Although artificially produced temperature elevations during winter months may actually bring the temperature closer to optimum or preferred temperature for important species and attract fish (Trembley 1965),[331] metabolic acclimation to these higher levels can preclude safe return of the organism to ambient temperatures should the artificial heating suddenly cease (Pennsylvania Fish Commission 1971;[310] Robinson 1970)[316] or the organism be driven from the heat area. For example, sockeye salmon (*Oncorhynchus nerka*) acclimated to 20 C suffered 50 percent mortality in the laboratory when their temperature was dropped suddenly to 5 C (Brett 1971:[256] see Figure III-3). The same population of fish withstood a drop to zero when acclimated to 5 C. The lower limit of the range of thermal tolerance of important species must, therefore, be maintained at the normal seasonal ambient temperatures throughout cold seasons, unless special provisions are made to assure that rapid temperature drop will not occur or that organisms cannot become acclimated to elevated temperatures. This can be accomplished by limitations on temperature elevations in such areas as discharge canals and mixing zones where organisms may reside, or by insuring that maximum temperatures occur only in areas not accessible to important aquatic life for lengths of time sufficient to allow metabolic acclimation. Such inaccessible areas would include the high-velocity zones of diffusers or screened discharge channels. This reduction of maximum temperatures would not preclude use of slightly warmed areas as sites for intense winter fisheries.

This consideration may be important in some regions at times other than in winter. The Great Lakes, for example, are susceptible to rapid changes in elevation of the thermocline in summer which may induce rapid decreases in shoreline temperatures. Fish acclimated to exceptionally high temperatures in discharge canals may be killed or severely stressed without changes in power plant operations (Robinson 1968).[314] Such regions should take special note of this possibility.

Some numerical values for acclimation temperatures and lower limits of tolerance ranges (lower incipient lethal temperatures) are given in Appendix II–C. Other data must be provided by further research. There are no adequate data available with which to estimate a safety factor for no stress from cold shocks. Experiments currently in progress, however, suggest that channel catfish fingerlings are more susceptible to predation after being cooled more than 5 to 6 C (Coutant, *unpublished data*).[324]

The effects of limiting ice formation in lakes and rivers should be carefully observed. This aspect of maximum winter temperatures is apparent, although there is insufficient evidence to estimate its importance.

Recommendation

Important species should be protected if the maximum weekly average temperature during winter months in any area to which they have access does not exceed the acclimation temperature (minus a 2 C safety factor) that raises the lower lethal threshold temperature of such species above the normal ambient water temperatures for that season, and the criterion for short-term exposures is not exceeded. This recommendation applies especially to locations where organisms may be attracted from the receiving water and subjected to rapid thermal drop, as in the low velocity areas of water diversions (intake or discharge), canals, and mixing zones.

SHORT-TERM EXPOSURE TO EXTREME TEMPERATURE

To protect aquatic life and yet allow other uses of the water, it is essential to know the lengths of time organisms can survive extreme temperatures (i.e., temperatures that exceed the 7-day incipient lethal temperature). Both natural environments and power plant cooling systems can briefly reach temperature extremes (both upper and lower) without apparent detrimental effect to the aquatic life (Fry 1951;[277] Becker et al. 1971).[243]

The length of time that 50 per cent of a population will survive temperature above the incipient lethal temperature can be calculated from a regression equation of experimental data (such as those in Figure III-3) as follows:

$$\log(\text{time}) = a + b\,(\text{temp.}) \qquad (\textit{Equation 2})$$

where time is expressed in minutes, temperature in degrees centigrade and where a and b are intercept and slope, respectively, which are characteristics of each acclimation temperature for each species. In some cases the time-temperature relationship is more complex than the semilogarithmic model given above. Equation 2, however, is the most applicable, and is generally accepted by the scientific community (Fry 1967).[278] Caution is recommended in extrapolating beyond the data limits of the original research (Appendix II–C). The rate of temperature change does not appear to alter this equation, as long as the change occurs more rapidly than over several days (Brett 1941;[253] Lemke 1970).[illegible] Thermal resistance may be diminished by the simultaneous presence of toxicants or other debilitating factors (Ebel et al. 1970,[273] and summary by Coutant 1970c).[269] The most accurate predictability can be derived from data collected using water from the site under evaluation.

Because the equations based on research on thermal tolerance predict 50 per cent mortality, a safety factor is needed to assure no mortality. Several studies have indicated that a 2 C reduction of an upper stress temperature results in no mortalities within an equivalent exposure duration (Fry et al. 1942;[illegible] Black 1953).[245] The validity of a two degree safety factor was strengthened by the results of Coutant (1970a).[261] He showed that about 15 to 20 per cent of the exposure time, for median mortality at a given high temperature, induced selective predation on thermally shocked salmon and trout. (This also amounted to reduction of the effective stress temperature by about 2 C.) Unpublished data from subsequent predation experiments showed that this reduction of about 2 C also applied to the incipient lethal temperature. The level at which there is no increased vulnerability to predation is the best estimate of a no-stress exposure that is currently available. No similar safety factor has been explored for tolerance of low temperatures. Further research may determine that safety factors, as well as tolerance limits, have to be decided independently for each species, life stage, and water quality situation.

Information needed for predicting survival of a number of species of fish and invertebrates under short-term conditions of heat extremes is presented in Appendix II–C. This information includes (for each acclimation temperature) upper and lower incipient lethal temperatures: coefficients a and b for the thermal resistance equation; and information on size, life stage, and geographic source of the species. It is clear that adequate data are available for only a small percentage of aquatic species, and additional research is necessary. Thermal resistance information should be obtained locally for critical areas to account for simultaneous presence of toxicants or other debilitating factors, a consideration not reflected in Appendix II-C data. More data are available for upper lethal temperatures than for lower.

The resistance time equation, Equation 2, can be rearranged to incorporate the 2 C margin of safety and also to define conditions for survival (right side of the equation less than or equal to 1) as follows:

$$1 \geq \frac{\text{time}}{10^{[a+b(\text{temp.}+2)]}} \qquad (\textit{Equation 3})$$

Low levels of mortality of some aquatic organisms are not necessarily detrimental to ecosystems, because permissible mortality levels can be established. This is how fishing or shellfishing activities are managed. Many states and international agencies have established elaborate systems for setting an allowable rate of mortality (for sport and commercial fish) in order to assure needed reproduction and survival. (This should not imply, however, that a form of pollution should be allowed to take the entire harvestable yield.) Warm discharge water from a power plant may sufficiently stimulate reproduction of some organisms (e.g., zooplankton), such that those killed during passage through the maximally heated areas are replaced within a few hours, and no impact of the mortalities can be found in the open water (Churchill and Wojtalik 1969;[168] Heinle 1969).[188] On the other hand, Jensen (1971)[393] calculated that even five percent additional mortality of 0-age brook trout (*Salvelinus fontinalis*) decreased the yield of the trout fishery, and 50 per cent additional mortality would, theoretically, cause extinction of the population. Obviously, there can be no adequate generalization concerning the impact of short-term effects on entire ecosystems, for each case will be somewhat different. Future research must be directed toward determining the effects of local temperature stresses on population dynamics. A complete discussion will not be attempted here. Criteria for complete short-term protection may not always be necessary and should be applied with an adequate understanding of local conditions.

Recommendation

Unless there is justifiable reason to believe it unnecessary for maintenance of populations of a species, the right side of Equation 3 for that species should not be allowed to increase above unity when the temperature exceeds the incipient lethal temperature minus 2 C:

$$1 \geq \frac{\text{time}}{10^{[a+b(\text{temp.}+2)]}}$$

Values for *a* and *b* at the appropriate acclimation temperature for some species can be obtained from Appendix II–C or through additional research if necessary data are not available. This recommendation applies to all locations where organisms to be protected are exposed, including areas within mixing zones and water diversions such as power station cooling water.

REPRODUCTION AND DEVELOPMENT

The sequence of events relating to gonad growth and gamete maturation, spawning migration, release of gametes, development of the egg and embryo, and commencement of independent feeding represents one of the most complex phenomena in nature, both for fish (Brett 1970)[344] and invertebrates (Kinne 1970).[396] These events are generally the most thermally sensitive of all life stages. Other environmental factors, such as light and salinity, often seasonal in nature, can also profoundly affect the response to temperature (Wiebe 1968).[323] The general physiological state of the organisms (e.g., energy reserves), which is an integration of previous history, has a strong effect on reproductive potential (Kinne 1970).[396] The erratic sequence of failures and successes of different year classes of lake fish attests to the unreliability of natural conditions for providing optimum reproduction.

Abnormal, short-term temperature fluctuations appear to be of greatest significance in reduced production of juvenile fish and invertebrates (Kinne, 1963).[394] Such thermal fluctuations can be a prominent consequence of water use as in hydroelectric power (rapid changes in river flow rates), thermal electric power (thermal discharges at fluctuating power levels), navigation (irregular lock releases), and irrigation (irregular water diversions and wasteway releases). Jaske and Synoground (1970)[392] have documented such temperature changes due to interacting thermal and hydroelectric discharges on the Columbia River.

Tolerable limits or variations of temperature change throughout development, and particularly at the most sensitive life stages, differ among species. There is no adequate summary of data on such thermal requirements for successful reproduction. The data are scattered through many years of natural history observations (however, see Breder and Rosen 1966[340] for a recent compilation of some data; also see Table III–13). High priority must be assigned to summarizing existing information and obtaining that which is lacking.

Uniform elevations of temperature by a few degrees during the spawning period, while maintaining short-term temperature cycles and seasonal thermal patterns, appear to have little overall effect on the reproductive cycle of resident aquatic species, other than to advance the timing for spring spawners or delay it for fall spawners. Such shifts are often seen in nature, although no quantitative measurements of reproductive success have been made in this connection. For example, thriving populations of many fishes occur in diverse streams of the Tennessee Valley in which the date of the spawning temperature may vary in a

TABLE III-13—Spawning Requirements of Some Fish, Arranged in Ascending Order of Spawning Temperatures (Adapted from Wojtalik, T. A., unpublished manuscript)*

Fishes	Temp. (C)	Spawning site	Range in spawning depth	Daily spawning time	Egg site	Incubation period days (Temp. C)
Sauger Stizostedion canadense	5.0	Shallow gravel bars	2-4 feet	Night	Bottom	25 (5.0)
Walleye S. vitreum vitreum	7.0	Gravel, rubble, boulders on bar	3-10 feet	Day, night	Bottom	....
Longnose gar Lepisosteus osseus	10.0	Flooded shallows	Flooded shallows	Day	Weeds	6 (20.0)
White bass Morone chrysops	11.7	Sand & rock shores	3-12 feet	Day, long but esp. night	Surface	2 (15.6)
Least darter Etheostoma microperca	12.6					
Spotted sucker Minytrema melanops	12.8					
White sucker Catostomus commersoni	12.0-13.6	Streams or bars	....	Day, night	Bottom	....
Silvery minnow Hybognathus nuchalis	13.0	Coves	....	Day	Bottom	....
Banded pygmy sunfish Elassoma zonatum	13.9-16.7					
White crappie Pomoxis annularis	14.0-16.0	Submerged materials in shallows	....	Day	Bottom	1 (21.1-23.2)
Fathead minnow Pimephales promelas	14.4 23.0	Shallows	Nr. surface	Day	Underside floating objects	....
Bigmouth buffalo Ictiobus cyprinellus	15.6-18.3	Shallows	....	Day	Bottom	9-10 (16.7)
Largemouth bass Micropterus salmoides	15.6	Shallows near bank	20 inches	Day	Bottom	5 (18.9)
Common shiner Notropis cornutus	15.6-18.3	Small gravel streams	....	Day	Bottom	
Golden shiner Notemigonus crysoleucas	15.6	Bays & shoals, weeds	....	Day	Weeds	4 (15.0*)
Green sunfish Lepomis cyanellus	15.6	Bank, shallows	Inches to 1½ feet	Day	Bottom	....
Paddlefish Polyodon spathula	16.0	Over gravel bars	Nr. surface	Night, day	Bottom	....
Blackside darter Percina maculata	16.5					
Gizzard shad Dorosoma cepedianum	16.7					
Smallmouth bass Micropterus dolomieui	16.7	Gravel rock shore	3-20 feet	Day	Bottom	7 (15.6)
Spotted bass Micropterus punctulatus	17.8	Small streams, bar	....	Day	Bottom	4-5 (20.0)
Johnny darter Etheostoma nigrum	18.0					
Orange spotted sunfish Lepomis humilis	18.3					
Smallmouth buffalo Ictiobus bubalus	18.9					
Black buffalo I. niger	18.9					
Carp Cyprinus carpio	19.0	Flooded shallows	Nr. surface	Day night	Bottom	4-8 (16.7)
Bluegill Lepomis macrochirus	19.4	Weeds, shallows	2-6 feet	Day	Bottom	1½-3 (22.2)
Redbreast sunfish L. auritus	20.0					
Channel catfish Ictalurus punctatus	21.0 26.7	Bank cavity	<10 feet	Day, night	Bottom	6-10 (15.6)
White catfish I. catus	20.0	Sand gravel bar	<10 feet	Day	Bottom	6-7 (23.9-26.4)
Pumpkinseed Lepomis gibbosus	20.0	Bank shallows	<5 feet	Day	Bottom	3 (27.8)
Black crappie Pomoxis nigromaculatus	20.0					
Brook silverside Labidesthes sicculus	20.0	Over gravel	Surface	Day	Weeds, bottom	....
Brown bullhead Ictalurus nebulosus	21.1	Shallows, weeds	Inches to 6 feet	....	Weeds, bottom	5 (25.0)
Threadfin shad Dorosoma petenense	21.1	Shallow and open water	Surface	Day	Bottom	3 (26.7)
Warmouth Lepomis gulosus	21.6	Bank shallows	<5 feet	Day	Bottom	1½ (25.0-26.7)
River redhorse Moxostoma carinatum	21.7-26.4	Riffles, streams	....	Day	Bottom	....

TABLE III-13—Spawning Requirements of Some Fish, Arranged in Ascending Order of Spawning Temperatures—Continued

Fishes	Temp. (C)	Spawning site	Range in spawning depth	Daily spawning time	Egg site	Incubation period days (Temp. C)
Blue catfish Ictalurus furcatus	22.2					
Flathead catfish Pylodictis olivaris	22.2					
Redear sunfish Lepomis microlophus	23.0	Quiet, various	Inches to 10 feet	....	....	....
Longear sunfish L. megalotis	23.3					
Freshwater drum Aplodinotus grunniens	23.0					
River carpsucker Carpiodes carpio	23.9					
Spotted bullhead Ictalurus serracanthus	26.7					
Yellow bullhead I. natalis	....	Quiet, shallows	1½–4 feet	....	Bottom	5–10 (18.9)

* T. A. Wojtalik, Tennessee Valley Authority, Muscle Shoals, Alabama.[illegible]

given year by 22 to 65 days. Examination of the literature shows that shifts in spawning dates by nearly one month are common in natural waters throughout the U.S. Populations of some species at the southern limits of their distribution are exceptions, e.g., the lake whitefish (*Coregonus clupeaformis*) in Lake Erie that require a prolonged, cold incubation period (Lawler 1965)[299] and species such as yellow perch (*Perca flavescens*) that require a long chill period for egg maturation prior to spawning (Jones, *unpublished data*).[327]

This biological plasticity suggests that the annual spring rise, or fall drop, in temperature might safely be advanced (or delayed) by nearly one month in many regions, as long as the thermal requirements that are necessary for migration, spawning, and other activities are not eliminated and the necessary chill periods, maturation times, or incubation periods are preserved for important species. Production of food organisms may advance in a similar way, with little disruption of food chains, although there is little evidence to support this assumption (but see Coutant 1968;[268] Coutant and Steele 1968;[271] and Nebeker 1971).[307] The process is similar to the latitudinal differences within the range of a given species.

Highly mobile species that depend upon temperature synchrony among widely different regions or environments for various phases of the reproductive or rearing cycle (e.g., anadromous salmonids or aquatic insects) could be faced with dangers of dis-synchrony if one area is warmed, but another is not. Poor long-term success of one year class of Fraser River (British Columbia) sockeye salmon (*Oncorhynchus nerka*) was attributed to early (and highly successful) fry production and emigration during an abnormally warm summer followed by unsuccessful, premature feeding activity in the cold and still unproductive estuary (Vernon 1958).[322] Anadromous species are able, in some cases, (see studies of eulachon (*Thaleichthys pacificus*) by Smith and Saalfeld 1955)[317] to modify their migrations and spawning to coincide with the proper temperatures whenever and wherever they occur.

Rates of embryonic development that could lead to premature hatching are determined by temperatures of the microhabitat of the embryo. Temperatures of the microhabitat may be quite different from those of the remainder of the waterbody. For example, a thermal effluent at the temperature of maximum water density (approximately 4 C) can sink in a lake whose surface water temperature is colder (Hoglund and Spigarelli, 1972).[290] Incubating eggs of such species as lake trout (*Salvelinus namaycush*) and various coregonids on the lake bottom may be intermittently exposed to temperatures warmer than normal. Hatching may be advanced to dates that are too early for survival of the fry in their nursery areas. Hoglund and Spigarelli 1972,[290] using temperature data from a sinking plume in Lake Michigan, theorized that if lake herring (*Coregonus artedii*) eggs had been incubated at the location of one of their temperature sensors, the fry would have hatched seven days early. Thermal limitations must, therefore, apply at the proper location for the particular species or life stage to be protected.

Recommendations

After their specific limiting temperatures and exposure times have been determined by studies tailored to local conditions, the reproductive activity of selected species will be protected in areas where:

- **periods required for gonad growth and gamete maturation are preserved;**
- **no temperature differentials are created that block spawning migrations, although some delay or advancement of timing based upon local conditions may be tolerated;**

- **temperatures are not raised to a level at which necessary spawning or incubation temperatures of winter-spawning species cannot occur;**
- **sharp temperature changes are not induced in spawning areas, either in mixing zones or in mixed water bodies (the thermal and geographic limits to such changes will be dependent upon local requirements of species, including the spawning microhabitat, e.g., bottom gravels, littoral zone, and surface strata);**
- **timing of reproductive events is not altered to the extent that synchrony is broken where reproduction or rearing of certain life stages is shown to be dependent upon cyclic food sources or other factors at remote locations.**
- **normal patterns of gradual temperature changes throughout the year are maintained.**

These requirements should supersede all others during times when they apply.

CHANGES IN STRUCTURE OF AQUATIC COMMUNITIES

Significant change in temperature or in thermal patterns over a period of time may cause some change in the composition of aquatic communities (i.e., the species represented and the numbers of individuals in each species). This has been documented by field studies at power plants (Trembley 1956–1960)[321] and by laboratory investigations (McIntyre 1968).[333] Allowing temperature changes to alter significantly the community structure in natural waters may be detrimental, even though species of direct importance to man are not eliminated.

The limits of allowable change in species diversity due to temperature changes should not differ from those applicable to any other pollutant. This general topic is treated in detail in reviews by others (Brookhaven National Lab. 1969)[255] and is discussed in Appendix II–B, Community Structure and Diversity Indices, p. 408.

NUISANCE ORGANISMS

Alteration of aquatic communities by the addition of heat may occasionally result in growths of nuisance organisms provided that other environmental conditions essential to such growths (e.g., nutrients) exist. Poltoracka (1968)[311] documented the growth stimulation of plankton in an artificially heated small lake; Trembley (1965[321]) reported dense growths of attached algae in the discharge canal and shallow discharge plume of a power station (where the algae broke loose periodically releasing decomposing organic matter to the receiving water). Other instances of algal growths in effluent channels of power stations were reviewed by Coutant (1970c).[269]

Changed thermal patterns (e.g., in stratified lakes) may greatly alter the seasonal appearances of nuisance algal growths even though the temperature changes are induced by altered circulation patterns (e.g., artificial destratification). Dense growths of plankton have been retarded in some instances and stimulated in others (Fast 1968;[275] and *unpublished data* 1971).[325]

Data on temperature limits or thermal distributions in which nuisance growths will be produced are not presently available due in part to the complex interactions with other growth stimulants. There is not sufficient evidence to say that any temperature increase will necessarily result in increased nuisance organisms. Careful evaluation of local conditions is required for any reasonable prediction of effect.

Recommendation

Nuisance growths of organisms may develop where there are increases in temperature or alterations of the temporal or spatial distribution of heat in water. There should be careful evaluation of all factors contributing to nuisance growths at any site before establishment of thermal limits based upon this response, and temperature limits should be set in conjunction with restrictions on other factors (see the discussion of Eutrophication and Nutrients in Section I).

CONCLUSIONS

Recommendations for temperature limits to protect aquatic life consist of the following two upper limits for any time of the year (Figure III–6).

1. One limit consists of a maximum weekly average temperature that:
   (a) in the warmer months (e.g., April through October in the North, and March through November in the South) is one third of the range between the optimum temperature and the ultimate upper incipient lethal temperature for the most sensitive important species (or appropriate life stage) that is normally found at that location at that time; or
   (b) in the cooler months (e.g., mid-October to mid-April in the North, and December to February in the South) is that elevated temperature from which important species die when that elevated temperature is suddenly dropped to the normal ambient temperature, with the limit being the acclimation temperature (minus a 2 C safety factor), when the lower incipient lethal temperature equals the normal ambient water temperature (in some regions this limit may also be applicable in summer); or
   (c) during reproduction seasons (generally April-June and September-October in the North, and March-May and October-November in the South) is that

temperature that meets specific site requirements for successful migration, spawning, egg incubation, fry rearing, and other reproductive functions of important species; or

d) at a specific site is found necessary to preserve normal species diversity or prevent undesirable growths of nuisance organisms.

2. The second limit is the time-dependent maximum temperature for short exposures as given by the species-specific equation:

$$1 \geq \frac{\text{time}}{10^{[a+b(\text{temp.}+2)]}}$$

Local requirements for reproduction should supersede all other requirements when they are applicable. Detailed ecological analysis of both natural and man-modified aquatic environments is necessary to ascertain when these requirements should apply.

USE OF TEMPERATURE CRITERIA

A hypothetical electric power station using lake water for cooling is illustrated as a typical example in Figure III-7. This discussion concerns the application of thermal criteria to this typical situation.

The size of the power station is 1,000 megawatts electric (MW_e) if nuclear, or 1,700 MW_e if fossil-fueled (oil, coal, gas); and it releases 6.8 billion British Thermal Units (BTU) per hour to the aquatic environment. This size is representative of power stations currently being installed. Temperature rise at the condensers would be 20 F with cooling water flowing at the rate of 1,520 cubic feet second (ft^3/sec) or 682,000 gallons/minute. Flow could be increased to reduce temperature rise.

The schematic Figure III-7 is drawn with two alternative discharge arrangements to illustrate the extent to which design features affect thermal impacts upon aquatic life

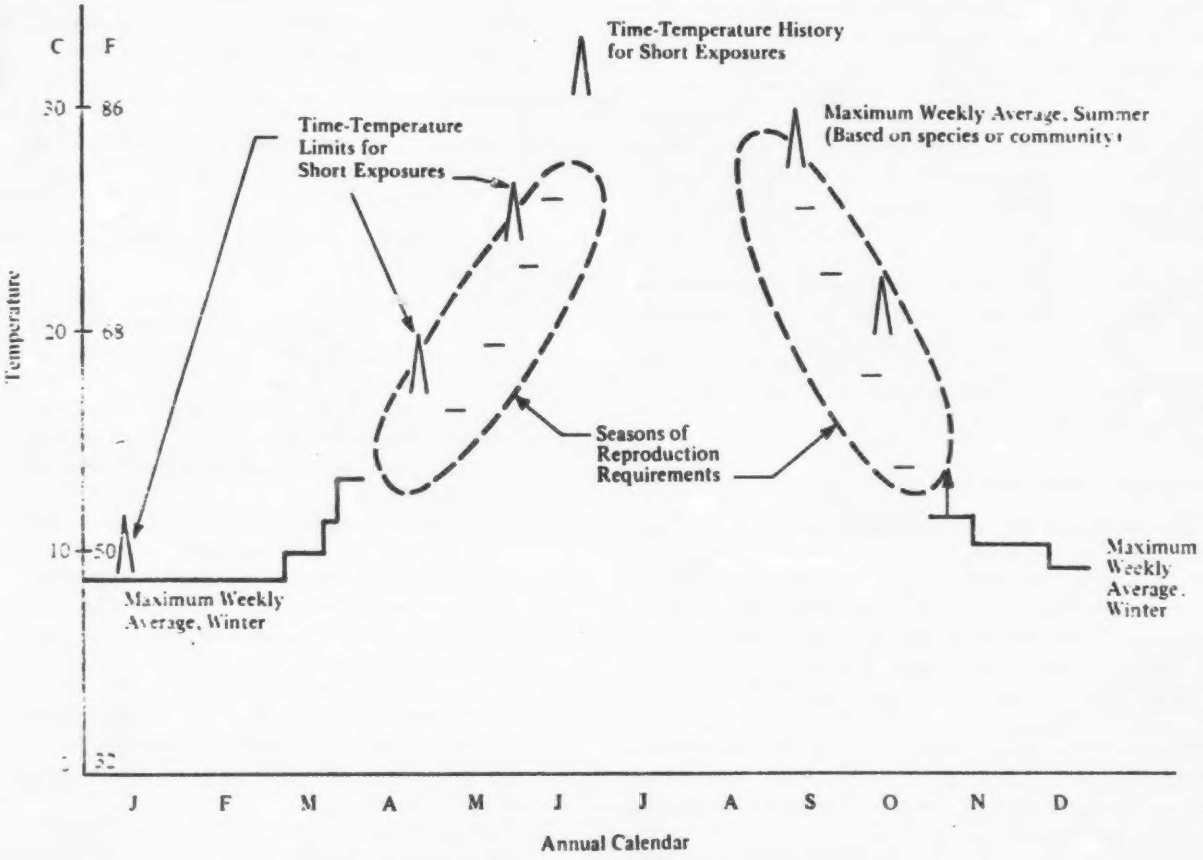


FIGURE III-6—Schematic Summary of Thermal Criteria

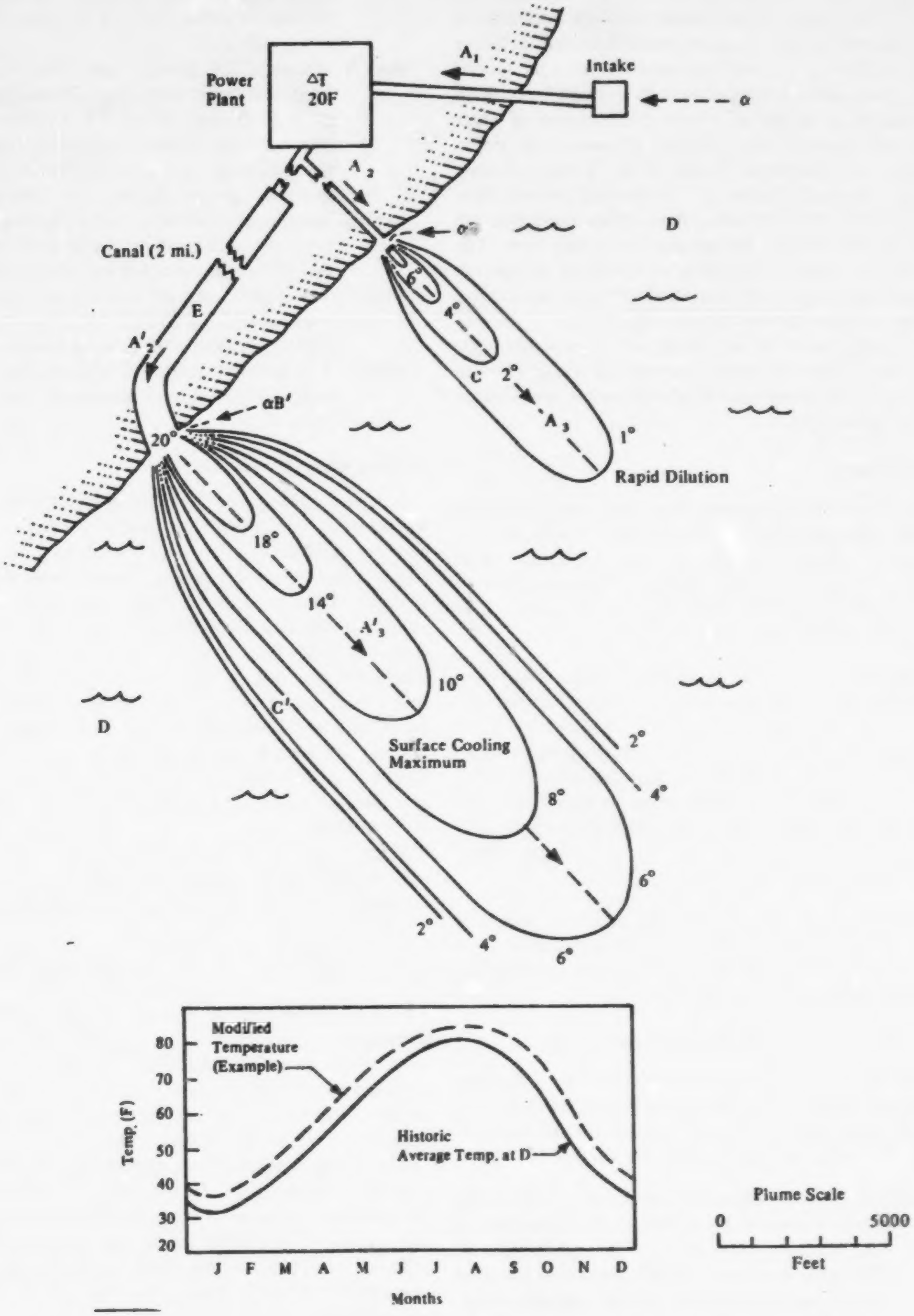

Power Plant
ΔT 20F
A1
Intake
α
A2
αB
D
Canal (2 mi.)
E
A'2
αB'
20°
6°
4°
C
2°
A3
1°
Rapid Dilution
18°
14°
A'3
10°
C'
D
Surface Cooling Maximum
2°
4°
8°
6°
2°
4°
6°
Modified Temperature (Example)
Historic Average Temp. at D
Temp. (F)
80
70
60
50
40
30
20
J F M A M J J A S O N D
Months
Plume Scale
0
5000
Feet

FIGURE III-7—Hypothetical Power Plant Site For Application of Water Temperature Criteria

Warm condenser water can be carried from the station to the lake by (a) a pipe carrying water at a high flow velocity or (b) a canal in which the warm water flows slowly. There is little cooling in a canal, as measurements at several existing power stations have shown. Water can be released to the lake by using any of several combinations of water velocity and volume (i.e., number of outlets) or outlet dimensions and locations. These design features largely determine the configuration of the thermal plumes illustrated in Figure III-7 resulting from either rapid dilution with lake water or from slow release as a surface layer. The isotherms were placed according to computer simulation of thermal discharges (Pritchard 1971)[312] and represent a condition without lake currents to aid mixing.

Exact configuration of an actual plume depends upon many factors (some of which change seasonally or even hourly) such as local patterns of currents, wind, and bottom and shore topography.

Analytical Steps

Perspective of the organisms in the water body and of the pertinent non-biological considerations (chemical, hydrological, hydraulic) is an essential beginning. This perspective requires a certain amount of literature survey or on site study if the information is not well known. Two steps are particularly important:

1. identification of the important species and community (primary production, species diversity, etc.) that are relevant to this site; and
2. determination of life patterns of the important species (seasonal distribution, migrations, spawning areas, nursery and rearing areas, sites of commercial or sport fisheries). This information should include as much specific information on thermal requirements as it is possible to obtain from the literature.

Other steps relate the life patterns and environmental requirements of the biota to the sources of potential thermal damage from the power plant. These steps can be identified with specific areas in Figure III-7.

Aquatic Areas Sensitive to Temperature Change

Five principal areas offer potential for biological damage from thermal changes, labeled A-E on Figure III-7. (There are other areas associated with mechanical or chemical effects that cannot be treated here; see the index.)

Area A The cooling water as it passes through the intake, intake piping (A_1), condensers, discharge piping (A_2) or canal (A'_2), and thermal plume (A_3 or A'_3), carrying with it small organisms (such as phytoplankton, zooplankton, invertebrate larvae, and fish eggs or larvae). Organisms receive a thermal shock to the full 20 F above ambient temperature with a duration that depends upon the rate of water flow and the temperature drop in the plume.

Area B Water of the plume alone that entrains both small and larger organisms (including small fish) as it is diluted (B or B′). Organisms receive thermal shocks from temperatures ranging from the discharge to the ambient temperature, depending upon where they are entrained.

Area C Benthic environment where bottom organisms (including fish eggs) can be heated chronically or periodically by the thermal plume (C or C′).

Area D The slightly warmed mixed water body (or large segment of it) where all organisms experience a slightly warmer average temperature (D).

Area E The discharge canal in which resident or seasonal populations reside at abnormally high temperatures (E).

Cooling Water Entrainment

It is not adequate to consider only thermal criteria for water bodies alone when large numbers of aquatic organisms may be pumped through a power plant. The probability of an organism being pumped through will depend upon the ratio of the volume of cooling water in the plant to the volume in the lake (or to the volume passing the plant in a river or tidal fresh water). Tidal environments (both freshwater and saline) offer greater potential for entrainment than is apparent, since the same water mass will move back and forth past the plant many times during the lifetime of pelagic residence time of most organisms. Thermal shocks that could be experienced by organisms entrained at the hypothetical power station are shown in Figure III-8.

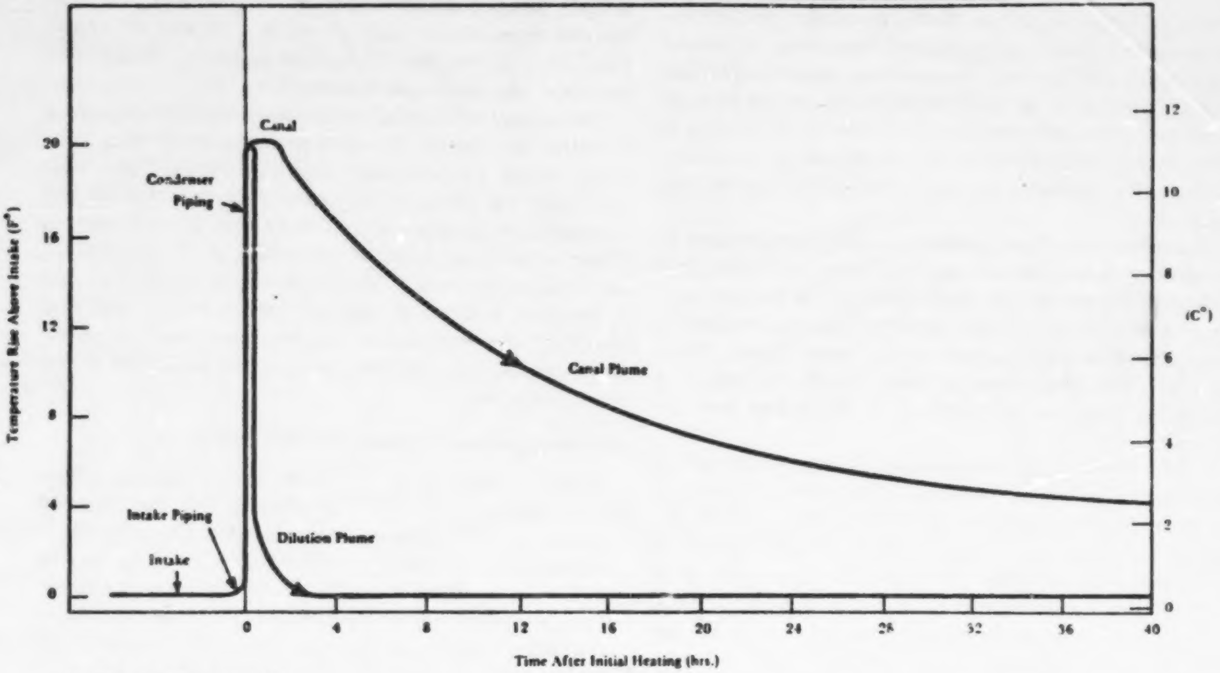
Detrimental effects of thermal exposures received during entrainment can be judged by using the following equation for short-term exposures to extreme temperatures:

$$\text{General criterion: } 1 \geq \frac{\text{time}}{10^{[a+b(\text{temp.}+2)]}}$$

Values for a and b in the equation for the species of aquatic organisms that are likely to be pumped with cooling water may be obtained from Appendix II, or the data may be obtained using the methods of Brett (1952).[314] The prevailing intake temperature would determine the acclimation temperature to be selected from the table.

For example, juvenile largemouth bass may frequent the near-shore waters of this lake and be drawn into the intake. To determine whether the hypothetical thermal discharges (Figure III-7) would be detrimental for juvenile bass, the following analysis can be made (assuming, for example, that the lake is in Wisconsin where these basic data for bass are available):

Criterion for juvenile bass (Wisconsin) when intake



Modified after Coutant 1970c[269]

FIGURE III-8—Time Course of Temperature Change in Cooling Water Passing Through the Example Power Station with Two Alternate Discharges. The Canal Is Assumed to Flow at a Rate of 3 Ft. Per Sec.

temperature (acclimation) is 70 F (21.11 C). (Data from Appendix II-C).

$$1 \geq \frac{\text{time}}{10^{[34.3649-0.9789(\text{temp.}+2)]}}$$

Canal

Criterion applied to entrainment to end of discharge canal (discharge temperature is 70 F plus the 20 degree rise in the condensers or 90 F (32.22 C). The thermal plume would provide additional exposure above the lethal threshold, minus 2 C (29.5 C or 85.1 F) of more than four hours.

$$1 \geq \frac{60}{10^{[34.3649-0.9789(32.22+2)]}}$$

$$1 \geq 8.15$$

Conclusion:

Juvenile bass would not survive to the end of the discharge canal.

Dilution

Criterion applied to entrainment in the system employing rapid dilution.

$$1 \geq \frac{1.2}{10^{[34.3649-0.9789(32.22+2.0)]}}$$

$$1 \geq \frac{1.2}{7.36}$$

Travel time in piping to discharge is assumed to be 1 min., and temperature drop to below the lethal threshold minus 2 C (29.5 C or 85.1 F) is about 10 sec. (Pritchard, 1971).[212]

Conclusion

Juvenile bass would survive this thermal exposure:

$$1 \geq 0.1630$$

By using the equation in the following form,

$$\log(\text{time}) = a + b\ (\text{temp.}+2)$$

the length of time that bass could barely survive the expected temperature rise could be calculated, thus allowing selection of an appropriate discharge system. For example:

$$\log(\text{time}) = 34.3649 - 0.9789\ (34.22)$$
$$\log(\text{time}) = 0.8669$$
$$\text{time} = 7.36$$

This would be about 1,325 feet of canal flowing at 3 ft/sec.

It is apparent that a long discharge canal, a nonrecirculating cooling pond, a very long offshore pipe, or delayed dilution in a mixing zone (such as the one promoting surface cooling) could prolong the duration of exposure of pumped organisms and thereby increase the likelihood of damage to them. Precise information on the travel times of the cooling water in the discharge system is needed to conduct this analysis.

The calculations have ignored changing temperatures in the thermal plume, because the canal alone was lethal, and cooling in the plume with rapid dilution was so rapid that the additional exposure was only for 10 seconds (assumed to be at the discharge temperature the whole time). There may be other circumstances under which the effect of decreasing exposure temperature in the plume may be of interest.

Effects of changing temperatures in the plume can be estimated by summing the effects of incremental exposures for short time periods (Fry et al. 1946[281]). For example, the surface cooling plume of Figures III–7 and III–8 could be considered to be composed of several short time spans, each with an average temperature, until the temperature had dropped to the upper lethal threshold minus 2 C for the juvenile bass. Each time period would be calculated as if it were a single exposure, and the calculated values for all time periods would be summed and compared with unity, as follows:

$$\frac{\text{time}_1}{10^{[a+b(\text{temp.}_1-2)]}} + \frac{\text{time}_2}{10^{[a+b(\text{temp.}_2+2)]}} + \cdots \frac{\text{time}_n}{10^{[a+b(\text{temp.}_n-2)]}}$$

The surface cooling plume of Figure III–6 (exclusive of the canal) could be considered to consist of 15 min at 89.7 F (32.06 C), 15 min at 89.2 F (31.78 C), 15 min at 88.7 F (31.4 C), 15 min at 88.2 F (31.22 C), 15 min at 87.8 F (31.00 C), until the lethal threshold for 70 F acclimation minus 2 C (85.1 F) was reached. The calculation would proceed as follows:

$$1 \geq \frac{15}{10^{[34.3649-0.9789(32.06+2)]}} + \frac{15}{10^{[34.3649-0.9789(31.78+2)]}} + \cdots$$

In this case, the bass would not survive through the first 15-minute period. In other such calculations, several steps would have to be summed before unity was reached (if not reached, the plume would not be detrimental).

Entrainment in the Plume

Organisms mixed with the thermal plume during dilution will also receive thermal shocks, although the maximum temperatures will generally be less than the discharge temperature. The number of organisms affected to some degree may be significantly greater than the numbers actually pumped through the plant. The route of maximum thermal exposure for each plume is indicated in Figure III–7 by a dashed line. This route should be analyzed to determine the maximum reproducible effect.

Detrimental effects of these exposures can also be judged by using the criterion for short-term exposures to extreme temperatures. The analytical steps were outlined above for estimating the effects on organisms that pass through the thermal plume portions of the entrainment thermal pattern. There would have been no mortalities of the largemouth bass from entrainment in the plume with rapid dilution, due to the short duration of exposure (about 10 seconds). Any bass that were entrained in the near-shore portions of the larger plume, and remained in it, would have died in less than 15 minutes.

Bottom Organisms Impacted by the Plume

Bottom communities of invertebrates, algae, rooted aquatic plants, and many incubating fish eggs can be exposed to warm plume water, particularly in shallow environments. In some circumstances the warming can be continuous, in others it can be intermittent due to changes in plume configuration with changes in currents, winds, or other factors. Clearly a thermal plume that stratifies and occupies only the upper part of the water column will have least effect on bottom biota.

Several approaches are useful in evaluating effects on the community. Some have predictive capability, while others are suitable largely for identifying effects after they have occurred. The criterion for short-term exposures identified relatively brief periods of detrimental high temperatures. Instead of the organism passing through zones of elevated temperatures, as in the previous examples, the organism is sedentary, and the thermal pulse passes over it. Developing fish eggs may be very sensitive to such changes. A brief pulse of high temperature that kills large numbers of organisms may affect a bottom area for time periods far longer than the immediate exposure time. Repeated sublethal exposures may also be detrimental, although the process is more complex than straight-forward summation. Analysis of single exposures proceeds exactly as described for plume entrainment.

The criterion for prolonged exposures is more generally applicable. The maximum tolerable weekly average temperature may be determined by the organisms present and the phase of their life cycle. In May, for example, the maximum heat tolerance temperature for the community may be determined by incubating fish eggs or fish fry on the bottom. In July it may be determined by the important resident invertebrate species. A well-designed thermal discharge should not require an extensive mixing zone where these criteria are exempted. Special criteria for reproductive processes may have to be applied, although thermal discharges should be located so that zones important for reproduction—migration, spawning, incubation—are not used.

Criteria for species diversity provide a useful tool for identifying effects of thermal changes after they have occurred, particularly the effects of subtle changes that are a result of community interactions rather than physiological responses by one or more major species. Further research may identify critical temperatures or sequences of temperature changes that cannot be exceeded and may thereby provide a predictive capability as well. (See Appendix II–B.)

Mixed Water Body (or major region thereof)

This is the region most commonly considered in establishing water quality standards, for it generally includes the major area of the water body. Here the results of thermal additions are observed as small temperature increases over a large area (instead of high temperatures locally at the discharge point), and all heat sources become integrated into the normal annual temperature cycle (Figure III–6 and Figure III–7 insert).

Detrimental high temperatures in this area (or parts of it) are defined by the criteria for maximum temperatures for prolonged exposure (warm and cool months) for the most sensitive species or life stage occurring there, at each time of year, and by the criteria for reproduction.

For example, in the lake with the hypothetical power station, there may be 40 principal fish species, of which half are considered important. These species have spawning temperatures ranging from 5 to 6 C for the sauger (*Stizostedion canadense*) to 26.7 C for the spotted bullhead (*Ictalurus serracanthus*). They also have a similar range of temperatures required for egg incubation, and a range of maximum temperatures for prolonged exposures of juveniles and adults. The requirements, however, may be met any time within normal time spans, such as January 1 to 24 for sauger spawning, and March 25 to April 29 for smallmouth bass spawning. Maximum temperatures for prolonged exposures may increase steadily throughout a spring period. To predict effects of thermal discharges the pertinent temperatures for reproductive activities and maximum temperatures for each life stage can be plotted over a 12-month period such as shown in Fig. III–6. A maximum annual temperature curve can become apparent when sufficient biological data are available. Mount (1970)[illegible] gives an example of this type of analysis.

Discharge Canal

Canals or embayments that carry nearly undiluted condenser cooling water can develop biological communities that are atypical of normal seasonal communities. Interest in these areas does not generally derive from concern for a balanced ecosystem, but rather from effects that the altered communities can have on the entire aquatic ecosystem.

The general criteria for nuisance organisms may be applicable. In the discharge canals of some existing power stations, extensive mats of temperature-tolerant blue-green algae grow and periodically break away, adding a decomposing organic matter to the nearby shorelines.

The winter criterion for maximum temperatures for prolonged exposures identifies the potential for fish kills due to rapid decreases in temperature. During cold seasons particularly, fish are attracted to warmer water of an enclosed area, such as a discharge canal. Large numbers may reside there for sufficiently long periods to become metabolically acclimated to the warm water. For any acclimation temperature there is a minimum temperature to which the species can be cooled rapidly and still survive (lower incipient lethal temperature). These numerical combinations, where data are available, are found in Appendix II–C. There would be 50 per cent mortality, for example, if largemouth bass acclimated in a discharge canal to 20 C, were cooled to 5.5 C or below. If normal winter ambient temperature is less than 5.5 C, then the winter maximum should be below 20 C, perhaps nearer 15 C. If it is difficult to maintain the lower temperatures, fish should be excluded from the area.

TOXIC SUBSTANCES

ORGANIC MERCURY

Until recently, mercury most commonly entered the aquatic environment by leaching from geological formations and by water transport to streams and lakes. Since the industrial revolution, however, increasing amounts of mercury have been added to the aquatic environment with waste products from manufacturing processes or through improper disposal of industrial and consumer products. In addition, large quantities of mercury enter the environment when ores are smelted to recover such metals as copper, lead, and zinc (Klein 1971),[343] and when fossil fuels are burned. Whereas the maximum amount of mercury released by weathering processes is approximately 230 metric tons per year worldwide, the amount released by the burning of coal is on the order of 3000 tons per year; and a further quantity, probably comparable to 3000 tons, is emitted from industrial processes (Joensuu 1971).[341]

In urban and industrial areas consumer products containing mercury are often disposed of in sewer systems. These mercury discharges, though individually small, cannot be considered insignificant, because cumulatively they add large quantities of mercury to the water courses that receive these effluents. On the average, the mercury concentration in sewage effluent is one order of magnitude greater than its concentration in the water course that receives it (D'Itri *unpublished data* 1971).[330] Based on Klein and Goldberg's 1970[344] report of mercury concentrations in samples of ocean sediments near municipal sewer out-falls, it can be calculated that in an urban area from 400 to 500 pounds of mercury per million population are discharged to receiving waters every year. The uses of mercury are varied, and its consumption is [illegible] large. The National Academy of Sciences (1969)[347] reported the consumption of mercury by user category.

World attention focused on the environmental mercury problem when human beings were poisoned by eating contaminated fish and shell fish during the middle and late 1950's in Minamata, Japan. Since the first occurrence of "Minamata disease" in 1953, 121 cases resulting in 46 deaths have been confirmed in the Minamata area with an additional 47 confirmed cases and 6 deaths in nearby Niigata (Takeuchi 1970).[352]

In Sweden in the 1950's, conservationists charged that the abundance of methylmercury in the environment was causing severe poisoning in seed-eating birds and their predators (Johnels et al. 1967).[340] These poisonings could be related to the use of methylmercury in seed dressings. When these seed dressings were prohibited, levels of mercury declined substantially in seed-eating animals. At about the same time, investigators found high levels of mercury in fish in waters off Sweden, practically all of it in the form of methylmercury.

Biological Methylation

Some microbes are capable of biologically synthesizing methylmercury from mercury ions (Jensen and Jernelöv 1969;[339] Wood et al. 1969;[353] Dunlap 1971;[333] Fagerström and Jernelöv 1971).[334] At low concentrations, the formation of dimethylmercury is favored in the methyl transfer reaction but at higher concentrations of mercury, the major product appears to be monomethylmercury. In any particular ecosystem, the amounts of mono- and dimethylmercury compounds are determined by the presence of microbial species, the amount of organic pollution loading, the mercury concentration, temperature, and pH (Wood et al. 1969).[353]

Biological Magnification

Aquatic organisms concentrate methylmercury in their bodies either directly from the water or through the food chain (Johnels et al. 1967;[340] Hannerz 1968;[336] Hasselrot 1968,[338] Miettinen et al. 1970[346]). Northern pike (*Esox lucius*) and rainbow trout (*Salmo gairdneri*) are able to assimilate and concentrate methylmercury directly into their muscle tissues from ingested food (Miettinen et al. 1970).[346] In general, mercury in organisms eaten by fish increases at each trophic level of the food chain (Hamilton 1971).[335] The magnitude of the bioaccumulation of mercury is determined by the species, its exposure, feeding habits, metabolic rate, age and size, quality of the water, and the degree of mercury pollution in the water. Rucker and

Amend (1969)[329] established that rainbow trout contained mercury levels of 4.0 and 17.3 μg/g in their muscle and kidney tissue after being exposed to 60 μg/l of ethylmercury for one hour a day over 10 days. Fresh water phytoplankton, macrophytes, and fish are capable of biologically magnifying mercury concentrations from water 1000 times (Chapman et al. 1968).[330] Johnels et al. (1967)[342] reported a mercury concentration factor from water to pike of 3000 or more. Johnels et al. (1967)[340] had previously shown that when mercury levels in pike muscle were below 0.2 μg/g, the level was relatively constant irrespective of weight, but above 0.2 μg/g, the concentration of mercury tended to increase with increasing age and weight.

Experiments in progress at the National Water Quality Laboratory in Duluth, Minnesota, (Mount *unpublished data* 1971)[347] indicate that when brook trout (*Salvelinus fontinalis*) are held in water containing 0.05 μg/l of methylmercury for 2 months they can accumulate more than 0.5 μg/g of mercury. This is a magnification of 10,000 times. In the same experiments, exposure to 0.03 μg/l for 5 months resulted in continuing accumulation in fish tissue with no indication of a plateau. In a group of fish held at one μg/l, some organs contained 30 μg/g. Some fresh water invertebrates have also been reported to have a 10,000 magnification (Hannerz 1968).[336]

Although the mechanisms by which mercury accumulates and concentrates have not been fully explained, at least three factors are involved: the metabolic rate of individual fish; differences in the selection of food as fish mature; and the epithelial surface of the fish (Wobeser et al. 1970;[357] Hannerz 1968).[336] The rate at which fish lose methylmercury also has considerable effect on magnification of mercury in the tissues. Miettinen et al. have shown in a series of papers (1970)[346] that the loss of methylmercury is both fast and slow in fishes. The fast loss occurs early, while mercury is being redistributed through the body, and lasts only a few weeks. The subsequent loss from established binding sites follows slowly; a half-life is estimated to be on the order of 2 years. These rates mean that fishes, and perhaps other lower vertebrates, reduce their content of methylmercury many times more slowly than do the higher terrestrial vertebrates. Man, for example, is usually considered to excrete half of any given mercury residue in about 80 days. Extremely low rates of loss have also been shown in different species of aquatic mollusks and crayfish (*Cambarus*) (Nelson 1971).[348]

Excessive mercury residues in the sediments are dissipated only slowly. Löfroth (1970)[345] estimated that aquatic habitats polluted with mercury continue to contaminate fish for as long as 10 to 100 years after pollution has stopped.

Mercury in Fresh Waters

Mercury measured in the water of selected rivers of the United States ranged from less than 0.1 μg/l to 17 μg/l. Two-thirds of the rivers contained 0.1 μg/l or less (Wallace et al. 1971).[355] The value of 0.1 μg/l is also reported as the earliest reliable estimate of mercury levels in uncontaminated fresh water (Swedish National Institute of Public Health 1971).[351] Some rivers tested by the Swedish Institute were as low as 0.05 μg/l, which was also the average mercury level in some salt waters.

Toxicity of Organic Mercury in Water

The chemical form of methylmercury administered to fish makes little difference in its toxic effect (Miettinen et al. 1970).[346] The methylmercury bound to sulfhydryl groups of proteins, as it would be in nature, is just as toxic as the free unbound ionic form.

Fish are able to survive relatively high concentrations of organomercurials for a short time with few ill effects. For example, fry of steel head trout (*Salmo gairdneri*) and fingerlings of sockeye salmon (*Oncorhynchus nerka*) are able to survive in 10 mg/l of pyridyl mercuric acetate for one hour with no toxic effects (Rucker and Whipple 1951).[350] The LC50 of pyridyl mercuric acetate for some freshwater fish ranges from 390 μg/l to 26,000 μg/l for exposures between 24 and 72 hours (Willford 1966;[356] Clemmens and Sneed 1958,[331] 1959).[332]

As the exposure times lengthen, lower concentrations of mercury are lethal. On the basis of 120-hour bioassay tests of three species of minnows, Van Horn and Balch (1955)[354] determined that the minimum lethal concentrations of pyridyl mercuric acetate, pyridyl mercuric chloride, phenyl mercuric acetate, and ethyl mercuric phosphate averaged 250 μg/l.

Recent experiments at the National Water Quality Laboratory (Mount, *personal communication* 1971)[347] indicated that 0.2 μg/l of methylmercury killed fathead minnows (*Pimephales promelas*) within 6 to 8 weeks. Toxicity data from this same laboratory on several other species including *Gammarus*, *Daphnia*, top minnow (*Fundulus* sp.) and brook trout (*Salvelinus fontinalis*) indicated that none was more sensitive than the fathead minnow.

Northern pike seem to be more sensitive. When they were reared in water containing 0.1 μg/l of methylmercury for a season and then placed in clean water, they underwent continuing mortality. Scattered mortality from this source could ordinarily not be detected in nature, because the affected fish became uncoordinated and probably would have been eaten by predators (Hannerz 1968,[336] quoted by Nelson 1971[348]).

Some species of plankton are particularly sensitive. Studies of the effect of mercury on phytoplankton species confirmed that concentrations as low as 0.1 μg/l of selected organomercurial fungicides decreased both the photosynthesis and the growth of laboratory cultures of the marine alga *Nitzschia delicatissima*, as well as of some fresh water phytoplankton species (Harriss et al. 1970).[337] Ethylmercury phosphate is lethal to marine phytoplankton at 60 μg/l, and levels as low as 0.5 μg/l drastically limit their growth (Ukeles 1962).[353] There is insufficient information about the thresholds for chronic toxicity.

Tissue Levels and Toxicity

There is almost no information on the concentrations of mercury in the tissues of aquatic organisms that are likely to cause mortality of the organisms themselves. Fish and shellfish found dead in Minamata contained 9 to 24 μg/g of mercury on the usual wet-weight basis; presumably some of these levels were lethal (Nelson 1971).[344] Miettinen et al. (1970)[345] showed that pike which had been experimentally killed by methylmercury contained from 5 to 9.1 μg/g and averaged 6.4 and 7.4 micrograms of methylmercury per gram of muscle tissue.

Discussion of Proposed Recommendations

At the present time there are not sufficient data available to determine the levels of mercury in water that are safe for aquatic organisms under chronic exposure. There have not been, for example, any experiments on the effects of chronic exposure to mercury on reproduction and growth of fish in the laboratory. Since experiments on sublethal effects are lacking, the next most useful information is on lethal effects following moderately long exposures of weeks or months. The lowest concentration shown to be lethal to fish is 0.2 μg/l of methylmercury which is lethal to fathead minnows (*Pimephales promelas*) in six weeks. Because 0.2 μg/l of methylmercury has been shown to be lethal, it is suggested that this concentration of mercury not be exceeded at any time or place in natural waters. Since phytoplankton are more sensitive, the average concentration of methylmercury in water probably should not exceed 0.05 μg/l for their protection. This recommended average is approximately equal to the supposed natural concentrations of mercury in water; hence little mercury can be added to the aquatic environment. The National Water Quality Laboratory (Mount, *unpublished data* 1971)[301] found that exposure of trout to 0.05 μg/l of methylmercury for 3 months resulted in concentrations of 0.5 μg/g, the Food and Drug Administration guideline for the maximum level for edible portions of fish flesh.

These concentrations of mercury or methylmercury in water are very low and difficult to measure or differentiate without special equipment and preparation. These low concentrations can also only be measured as total mercury. Since sediments may contain 10,000 times the amount of mercury in water, suspended solids in water can seriously affect the values found in analyses of water for mercury (Jernelöv 1972).[340] Because of these difficulties and because the real danger of mercury pollution results from a biological magnification, recommendations for mercury residues in tissues of aquatic organisms should be developed. This would make monitoring and control not only more effective and certain but also more feasible technically. Unfortunately, data are not yet available on the residue levels that are safe for the aquatic organisms themselves and for organisms higher in the food chain, such as predatory fish or fish-eating birds. It is known that concentrations of 5 to 10 μg/g are found in some fish that died of methylmercury poisoning, and that 0.01 to 0.2 μg/g is apparently a usual background level in freshwater fish. Because data are lacking for safe residue levels in aquatic food chains, it is suggested that the Food & Drug Administration guideline level of 0.5 μg/g of total mercury in edible portions of freshwater fish used as human food be the guideline to protect predators in aquatic food chains.

Hence, mercury residues should not exceed 0.5 μg/g in any aquatic organisms. If levels approaching this are found, there should be total elimination of all possible sources of mercury pollution.

No distinction has been drawn between organic and inorganic forms of mercury in these discussions because of the possibility of biological transformation to the organic phase in aquatic habitats. Since the form of mercury in water cannot be readily determined, the recommendations are primarily based upon methylmercury but expressed as total mercury.

Recommendations

Selected species of fish and predatory aquatic organisms should be protected when the following conditions are fulfilled: (1) the concentration of total mercury does not exceed a total body burden of 0.5 μg/g wet weight in any aquatic organism; (2) the total mercury concentrations in unfiltered water do not exceed 0.2 μg/l at any time or place; and (3) the average total mercury concentration in unfiltered water does not exceed 0.05 μg/l.

PHTHALATE ESTERS

The occurrence of dialkyl phthalate residues has been established in various segments of the aquatic environment of North America. Phthalate ester residues occur principally in samples of water, sediment, and aquatic organisms in industrial and heavily populated areas (Stalling 1972).[360] In fish di-*n*-butyl phthalate residues ranged from 0 to 500 μg/kg, and di-2-ethylhexyl phthalate residues were as high as 3,200 μg/kg. No well-documented information exists on the fate of phthalate compounds in aquatic environments.

Phthalate esters are widely used as plasticizers, particularly in polyvinyl chloride (PVC) plastics. The most common phthalate ester plasticizer is di-2-ethylhexyl phthalate. Di-*n*-butyl phthalate has been used as an insect repellent (Frear 1969)[362] and in pesticide formulations to retard volatilization (Schoof et al. 1963).[361] Production of dioctyl phthalate ester placticizers was estimated to be 4.10 × 10^5 lbs in 1970 (Neelyl 970).[363] Total phthalate ester production was reported to be 8.40 × 10^8 lbs in 1968, of which 4.40 × 10^8 lbs were dioctyl phthalate esters (Nematollahi et al. 1967).[364] Production of phthalic anhydride was estimated to be

7.60×10⁸ lbs in 1970 (Neely 1970).[383] PVC plastic formulations may contain 30 to 60 parts per hundred of phthalate ester plasticizer (Nematollahi et al. 1967).[384]

Toxicity

Studies to determine the acute or chronic toxicity effects of phthalate esters or other plasticizers on aquatic organisms have only recently been undertaken (Stalling 1972).[386] For example, the acute toxicity of di-*n*-butyl phthalate to fish is extremely low compared to pesticides (Table III-14).

Daphnia magna were exposed to 0.1 µg/l of ^{14}C di-*n*-butyl phthalate and the organisms accumulated chemical residues of 600 µg/kg within 10 days, or a 6,000-fold magnification (Saunders, *unpublished data* 1971).[387] However, after transfer of the *Daphnia* to uncontaminated water, approximately 50 per cent of the di-*n*-butyl phthalate was excreted in three days. It was recently found that a concentration of 3 µg/l of di-2-ethylhexyl phthalate significantly reduced the growth and reproduction of *Daphnia magna* (Sanders *unpublished data* 1971).[387]

The acute toxicity of phthalate esters appears to be relatively insignificant, but these compounds may be detrimental to aquatic organisms at low chronic concentrations.

Recommendation

Until a more detailed evaluation is made of toxicological effects of phthalate esters on aquatic ecosystems, a safety factor of 0.1 has been applied to data for *Daphnia magna* toxicity, and a level not to exceed 0.3 µg/l should protect fish and their food supply.

POLYCHLORINATED BIPHENYLS

Polychlorinated biphenyls (PCB) have been found in fish and wildlife in many parts of the world and at levels that may adversely affect aquatic organisms (Jensen et al. 1969;[374] Holmes et al. 1967;[375] Koeman et al. 1969;[376] Risebrough et al. 1968).[386] The environmental occurrence, uses, and present toxicological aspects of PCB were recently reviewed by Peakall and Lincer (1970),[384] Gustafson (1970),[373] Risebrough (1970),[387] and Reynolds (1971).[385]

TABLE III-14—Acute Toxicity of Di-n-butyl Phthalate to Four Species of Fish and Daphnia Magna.

Species	Temperature	LC50 in µg/l 24 hr	48 hr	96 hr
Fathead minnow (Pimephales promelas)			1400	1300 (Stalling)
Bluegill (Lepomis macrochirus)		1230	731	731 (Stalling)
Channel catfish (Ictalurus punctatus)		3720	2910	2910 (Stalling)
Rainbow trout (Salmo gairdneri)				6470 (Sanders)
Daphnia magna				>1000 (Sanders)

Biphenyls may have 1 to 10 attached chlorine atoms, making possible over 200 compounds (Gustafson 1970).[373] PCB occur as residues in fish, and presumably also in water, as mixtures of chlorinated biphenyl isomers as shown in Table III-15 (Stalling and Johnson, *unpublished data* 1970,[390] Stalling *in press*[389]).

Analysis of PCB has been accomplished by gas chromatography after separation of PCB from pesticides. A separation method has been described by Armour and Burke (1970)[380] and modified by Stalling and Huckins (1971).[391] A method using separation on a charcoal column has shown good reproducibility (Frank and Rees, *personal communication*).[392] No standardized gas-liquid chromatography method has been proposed for the analysis of mixtures of PCB in environmental samples. The solubility of these formulations in water has not been precisely determined, but it is in the range of 100 to 1,000 µg/l (Papageorge 1970).[383] Since PCB have gas chromatographic characteristics similar to many organochlorine pesticides, they can cause serious interference in the gas chromatographic determination of chlorinated insecticides (Risebrough et al. 1968).[386]

The Monsanto Company, the sole manufacturer of PCB

TABLE III-15—Composition of PCB Residues in Selected Fish Samples from the 1970 National Pesticide Residue Monitoring Program

River	Location	Species	PCB Residue as Aroclor® type (µg/g whole body) 1242	1248	1254	1260	Total
Ohio	Cincinnati, O.	Carp Cyprinus carpio	50	75	42	6.0	173
Ohio	Cincinnati, O.	White crappie Pomoxis annularis	18	17	27	6.0	68
Ohio	Marietta, O.	Channel catfish Ictalurus punctatus	38	23	11	4.9	77
Ohio	Marietta, O.	Channel catfish	16	5.2	13	4.6	38
Yazoo	Redwood, Miss.	Smallmouth buffalo Ictiobus bubalus	72		1.4		73
Hudson	Poughkeepsie, N.Y.	Goldfish Carassius auratus	9	173	30		212
Allegheny	Natrona, Pa.	Walleye Stizostedion vitreum v.		5.2	25	4.6	35
Delaware	Camden, N.J.	White perch Roccus americanus		8.0	6.6	3.9	19
Cape Fear	Elizabeth Town, N.C.	Gizzard shad Dorosoma cepedianum	19		2.6	1.1	23
Lake Ontario	Port Ontario, N.Y.	White perch	13		4.6	1.2	19
Mississippi	Memphis, Tenn.	Drum Aplodinotus grunniens	11		4.6	3.4	19
Merrimac	Lowell, Mass.	Drum	14	75	8.1	3.2	98

in the United States (Gustafson 1970),[373] markets eight formulations of chlorinated biphenyls under the trademarks Aroclor® 1221, 1232, 1242, 1248, 1254, 1260, 1262, and 1268. The last two digits of each formulation designate the percent chlorine. Aroclor® 1248 and 1254 are produced in greatest quantities. They are used as dielectric fluids in capacitors and in closed-system heat exchangers (Papageorge 1970).[384] Aroclor® 1242 is used as a hydraulic fluid, and Aroclor® 1260 as a plasticizer. Chlorinated terphenyls are marketed under the trademark Aroclor® 5442 and 5460, and a mixture of bi- and terphenyls is designated Aroclor® 4465. The isomer composition and chromatographic characteristics of each formulation have been described by Stalling and Huckins (1971)[391] and Bagley et al. (1970).[370] A contaminant of some PCB, especially those manufactured in Europe, are chlorinated dibenzofurans (Brungs *personal communication* 1972).[393] Although these byproducts would appear to be extremely toxic, no data are available on their toxicity to aquatic life.

Direct Lethal Toxicity

Studies of toxicity of PCB to aquatic organisms are limited. They show considerable variation of toxicity to different species, as well as variation with the chlorine content of the PCB. Nevertheless, some trends in the toxic characteristics have become apparent, principally from the work of Mayer as described below:

- The higher the per cent chlorine, the lower the apparent toxicity of PCB to fish (Mayer, *in press*).[379] This was found in 15-day intermittent-flow bioassays using bluegills (*Lepomis macrochirus*) and channel catfish (*Ictalurus punctatus*) with Aroclor® 1242, 1248, 1254. All LC50 values were in the range 10 to 300 μg/l.
- The bluegill/channel catfish experiments also illustrated that all LC50 values decreased significantly when exposures continued from 15 to 20 days. The 96-hour LC50 of a PCB to fish cannot adequately measure its lethal toxicity.
- The same tests showed that the toxicity of Aroclor® 1248 doubled when the temperature was raised from 20 C to 27 C.

To invertebrates, Aroclor® 1242 has about the same acute toxicity that it has to fish. In 4- and 7-day tests (Saunders, *in press*),[389] it killed *Gammarus* at 42 μg/l and crayfish (*Cambarus*) at 30 μg/l, with values that were similar to the 15-day LC50 reported for bluegills. However, there is an extreme range in the reported short-term lethal levels of Aroclor® 1254 for invertebrates. Saunders (*in press*)[389] reported a 96-hour LC50 as 80 μg/l for crayfish and only 3 μg/l for glass shrimp (*Paleomonetes*) in 7-day tests; and Duke et al. (1970)[371] reported that as little as 0.94 μg/l killed immature pink shrimp (*Panaeus duorarum*). Part of this variation is related to exposure periods in the tests; part is no doubt the variation in species response. Again this emphasizes the point that short-term tests of acute toxicities of PCB have serious limitations.

Marine animals may be more easily killed by PCB than freshwater ones (see Section IV). When two estuarine fishes (*Lagodon rhomboides* and *Leiostomus xanthurus*) were exposed for 14 to 45 days to Aroclor® 1254, mortalities were observed at 5 μg l (Hansen, et al. 1971).[373] This indicated a toxicity about five times greater than summarized above for freshwater fish but about the same as the toxicity for the marine crustaceans mentioned above.

Feeding Studies

Dietary exposure to PCB seems to be less of a direct hazard to fish than exposure in water. Coho salmon (*Oncorhynchus kisutch*) fed Aroclor® 1254 in varying amounts up to 14,500 μg/kg body weight per day accumulated whole body residues which were only 0.9 to 0.5 of the level in the food after 240 days of dietary exposure. Growth rates were not affected. However, all fish exposed to the highest treatment died after 240 days exposure; and thyroid activity was stimulated in all except the group treated at the lowest concentration (Mehrle and Grant *unpublished data* 1971).[394]

At present, evaluation of data from laboratory experiments indicates that exposures to PCB in water represents a greater hazard to fish than dietary exposures. However, in the environment, residue accumulation from dietary sources could be more important, because PCB have a high affinity for sediments, and therefore, they readily enter food chains (Duke et al. 1970;[371] Nimmo, et al. 1971).[382]

Residues in Tissue

It is clear that widespread pollution of major waterways has occurred, and that appreciable PCB residues exist in fish. When analyses of 40 fish from the 1970 National Pesticide Monitoring Program were made, only one of the fish was found to contain less than 1 μg/g PCB (Stalling and Mayer 1972).[390] The 10 highest residue levels in the 40 selected fish ranged from 19 μg/g to 213 μg/g whole body weight.

By contrast, residues measured in ocean fish have been generally below 1 μg/g (Risebrough 1970;[387] Jensen, et al. 1969).[376] Between the ranges in freshwater fish and those in marine fish are the levels of PCB found in seals (Jensen et al. 1969;[376] Holden 1970),[374] and in the eggs of fish-eating birds in North America (Anderson et al. 1969;[365] Mulhern et al. 1971;[380] Reynolds 1971).[386]

In laboratory experiments, crustacea exposed to varying levels of Aroclor® 1254 in the water concentrated the PCB within their bodies more than 20,000 times. The tissue residues may sometimes reach an equilibrium, and in *Gammarus fasciatus* PCB did not concentrate beyond 27,000 times despite an additional 3-week exposure to 1.6 μg l Aroclor[2] (Saunders 1972).[388] In contrast, PCB residues in crayfish did not reach equilibrium after a 28-day exposure.

PCB concentration factors by two estuarine fishes, *Lagondon rhomboides* and *Leiostomus xanthurus*, were similar to that described above for crustaceans, i.e., about 10,000 to 50,000 times the exposure levels in water (Hansen et al. 1971).[373] It is important to note that these accumulations occurred at water concentrations of PCB that killed the fish in 15 to 45 days.

Also similar were the accumulation ratios of 26,000 to 56,000 for bluegills (*Lepomis macrochirus*) chronically exposed to 2 to 15 μg/l of Aroclor® 1248 and 1254. Fathead minnows (*Pimephales promelas*) chronically exposed to Aroclor® 1242 and 1254 for 8 weeks concentrated PCB 100,000 and 200,000 times the exposure levels, respectively. Residues of 50 μg/l (whole body) resulted from exposure for 8 weeks to 0.3 μg/l Aroclor® 1254 (Nebeker et al. 1972).[381] These experiments with bluegills also indicated that the maximum levels of PCB were generally related to the concentration of PCB in the water (50,000–200,000 times higher) to which they were exposed (Stalling and Huckins *unpublished data* 1971).[397]

Effects on Reproduction

PCB residues in salmon eggs are apparently related to mortality of eggs. In preliminary investigations in Sweden, Jensen and his associates (1970)[377] reported that when residues in groups of eggs ranged from 0.4 to 1.9 μg/g on a whole-weight basis (7.7 to 34 μg/g on a fat basis), related mortalities ranged from 16 per cent up to 100 per cent.

PCB concentrations in the range of 0.5 to 10 μg/l in water interfered with reproduction of several aquatic animals according to recent work of Nebeker et al. (1971).[381] About 5 μg/l of Aroclor® 1248 was the highest concentration that did not affect reproduction of *Daphnia magna* and *Gammarus pseudolimnaeus*. In tests of reproduction by fathead minnows (*Pimephales promelas*) all died when exposed chronically to greater than 8.3 μg/l of either Aroclor® 1242 or Aroclor® 1254. Reproduction occurred at and below 5.4 μg/l Aroclor® 1242, and at and below 1.8 μg/l of Aroclor® 1254.

The association between residue levels and biological effects in aquatic animals is scarcely known, but the work of Jensen et al. (1970)[377] suggested that about 0.5 μg/g of PCB in whole salmon eggs might be the threshold for egg mortality. Such a level in eggs would be associated with levels in general body tissue (e.g., muscle) of 2.5 to 5.0 μg/g. The residue in muscle corresponded to the present Food and Drug Administration level for allowable levels of PCB in fish used as human food. Residues measured in the survey by the 1970 National Pesticide Monitoring Program were generally above 5 μg/g.

Applying a minimal safety factor of 10 for protection of the affected population, and for protection of other species higher in the food chain, would yield a maximum permissible tissue concentration of 0.5 μg/g in any aquatic organism in any habitat affected by PCB.

General Considerations and Further Needs

Another means of control would be justified in view of the toxicity of PCB, the lack of knowledge about how it first enters natural ecosystems as a pollutant, and its apparent distribution in high concentrations in freshwater fish in the United States. This method would be to regulate the manufacture of PCB and maintain close control of its uses to avoid situations where PCB is lost to the environment. The Monsanto Company recently restricted the sale of PCB for uses in which disposal of the end products could not be controlled, as with plasticizers (Gustafson 1970).[372]

Basis for Recommendations

For PCB levels in water, the most sensitive reaction shown by aquatic organisms is to the lethal effects of low concentrations continually present in water for long periods (weeks or months). Concentrations in the range of 1 to 8 μg/l have been shown to be lethal to several animals.

The work of Hansen, et al. (1971)[373] and Stallings and Huckins (*unpublished data* 1971)[391] indicates that concentrations of 0.01 μg/l of PCB in water over periods of up to 36 weeks could lead to dangerous levels of PCB in the tissues of aquatic organisms. Accumulation by factors of 75,000 to 200,000 times is indicated by their work. If the higher ratio is taken, 0.01 μg/l in water might result in 2.0 μg/g in flesh on whole fish basis. This is comparable to the residue level in salmon eggs associated with complete mortality of embryos. Therefore, a concentration is recommended that is reduced by a factor of 5, or 0.002 μg/l. In addition, a control based on residue levels is required, as well as one based on PCB in the water.

Recommendations

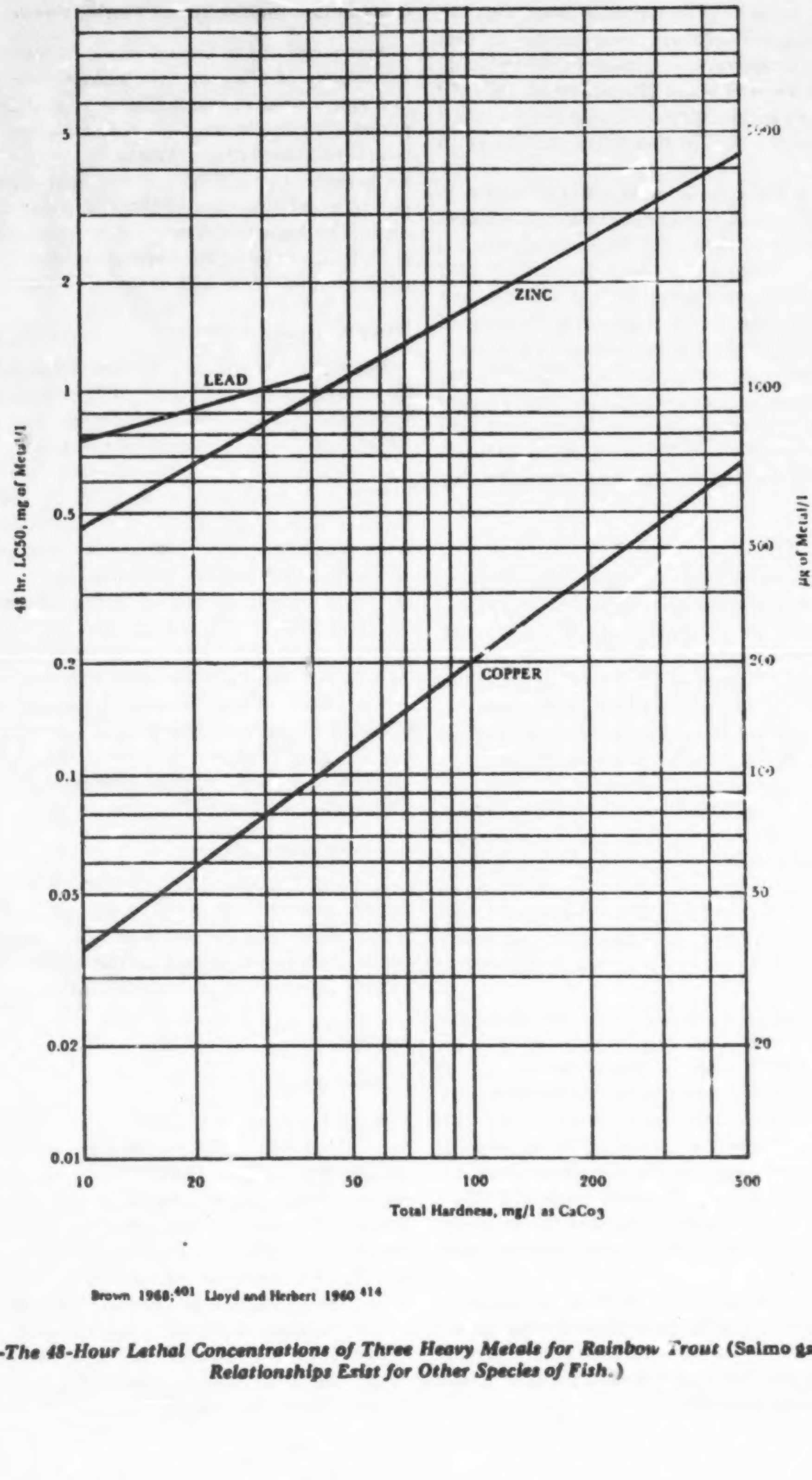
Aquatic life should be protected where the maximum concentration of total PCB in unfiltered water does not exceed 0.002 μg/l at any time or place, and the residues in the general body tissues of any aquatic organism do not exceed 0.5 μg/g.

METALS

General Data

Several reviews of the toxicity of metals are available (e.g., Skidmore 1964;[428] McKee and Wolf 1963;[415] Doudoroff and Katz 1953).[406] Some of the most relevant research is currently in progress or only recently completed. Some deals with chronic effects of metals on survival, growth, and reproduction of fish and other organisms. The completed studies have estimated safe concentrations, and from these application factors have been derived as defined in the discussion of bioassays (pp. 118–123).

The important relation between water hardness and lethal toxicity is well documented for some metals (see Figure III–9). For copper, the difference in toxicity may



Brown 1968;[401] Lloyd and Herbert 1960 [414]

FIGURE III-9—The 48-Hour Lethal Concentrations of Three Heavy Metals for Rainbow Trout **(Salmo gairdneri).** ***(Similar Relationships Exist for Other Species of Fish.)***

not be related to the difference in hardness per se, but to the difference in alkalinity of the water that accompanies change in hardness (Stiff 1971).[434] Nevertheless, the relation to hardness is a convenient and accepted one. The hardness classification developed by the U.S. Geological Survey is the following:

Soft	0–60 mg/l (hardness as $CaCO_3$)
Moderately hard	61–120 mg/l
Hard	in excess of 120 mg/l

There are many chemical species of metals in water; some are toxic to aquatic life, others are not. Hydrogen ion concentration in water is extremely important in governing the species and solubility of metals and therefore the lethal toxicity. At high pH, many heavy metals form hydroxides or basic carbonates that are relatively insoluble and tend to precipitate. They may, however, remain suspended in the water as fine particles (O'Connor et al. 1964;[421] Stiff 1971).[434]

The toxicity of suspended hydroxides of metal depends on the particular situation. For example, suspended zinc has been found to be nontoxic (Sprague 1964a&b),[429,430] equally as toxic as dissolved zinc (Lloyd 1960)[412] and more toxic than dissolved zinc (Mount 1966).[417] This indicates that suspended zinc is at least potentially poisonous, and therefore the total metal measured in the water should be considered toxic. It is difficult to predict the effect of pH on toxicity. For example, low pH (about 5) as well as high pH (about 9) reduced toxicity of copper and zinc compared to that at neutral pH (Fisheries Research Board of Canada *unpublished data* 1971).[444] Therefore pH should be regulated in bioassays with metals in order to simulate local conditions and to explore any effect of local variation of pH.

In addition to hardness, numerous other factors influence the lethal toxicity of copper to fish. McKee and Wolf (1963)[415] and Doudoroff and Katz (1953)[406] included dissolved oxygen, temperature, turbidity, carbon dioxide, magnesium salts, and phosphates as factors affecting copper toxicity. Artificial chelating compounds such as nitrilotriacetic acid can reduce or eliminate toxic effects of zinc and other metals (Sprague 1968b)[432] and there may be natural chelating agents that would do the same thing. Certain organic ligands (Bender et al. 1970)[399] and amino acids from sewage treatment plant effluent (United Kingdom Ministry of Technology 1969)[435] also reduce the toxicity of copper by forming copper-organic complexes that do not contribute to lethal toxicity. It is safe to assume that some of these factors will influence the toxicity of other metals. In addition, the amount of metals found (at least temporarily) in living biological matter is included in most routine water analyses. At the present time, however, it is not possible to predict accurately the amount of total metal in any environment that may be lethal, biologically active, or contributory to toxicity. Consequently, the following recommendations are made.

Recommendations

Since forms or species of metals in water may change with shifts in the water quality, and since the toxicity to aquatic life may concurrently change in as yet unpredictable ways, it is recommended that water quality criteria for a given metal be based on the total amount of it in the water, regardless of the chemical state or form of the metal, except that settleable solids should be excluded from the analysis (Standard Methods 1971).[433] Additionally, hardness affects the toxicity of many metals (see Figure III–9).

Metals which have collected in the sediments can redissolve into the water, and such redissolved metals should meet the criteria for heavy metals. To protect aquatic life, amounts likely to be harmful should not occur in the sediments.

It is recommended that any metal species not specifically mentioned in this report but suspected of causing detrimental effects on aquatic life be examined as outlined in the section on Bioassays.

Aluminum

Current research by Freeman and Everhart (1971)[407] indicated that aluminum salts were slightly soluble at neutral pH; 0.05 mg/l dissolved and had no sublethal effects on fish. At pH 9, at least 5 mg/l of aluminum dissolved and this killed fingerling rainbow trout within 48 hours. However, the suspended precipitate of ionized aluminum is toxic. In most natural waters, the ionized or potentially ionizable aluminum would be in the form of anionic or neutral precipitates, and anything greater than 0.1 mg/l of this would be deleterious to growth and survival of fish.

Recommendation

Careful examination of toxicity problems should be made to protect aquatic life in situations where the presence of ionic aluminum is suspected. Aluminum may have considerably greater toxicity than has been assumed.

Cadmium

This metal is an extremely dangerous cumulative poison. In mammals (Nilsson 1970),[420] fish (Eaton *unpublished data* 1971),[442] and probably other animals, there is insidious, progressive, chronic poisoning because there is almost no excretion of the metal. In its acute lethal action on rainbow trout (*Salmo gairdneri*), Ball (1967)[394] found cadmium unusually slow. A lethal threshold of 0.01 mg/l was not discernible until seven days' exposure. Other investigators (Pickering and Gast, *in press*,[427] Eaton *unpublished data* 1971)[442] have determined lethal threshold concentrations in fathead minnows in 2 to 6 days and in bluegill in 96 hours. The chronically safe levels for both fathead minnows

(*Pimephales promelas*) (Pickering and Gast, *in press*)[427] and bluegill sunfish (*Lepomis macrochirus*) (Eaton *unpublished data* 1971)[442] in hard water (200 mg/l as $CaCO_3$) are between 0.06 and 0.03 mg/l. In these exposures, death of eggs or early larvae was one of the effects observed at the lowest unsafe concentrations tested. Recent exposures of eggs and larvae at the National Water Quality Laboratory (Duluth) in soft water (45 mg/l as $CaCO_3$) demonstrated that 0.01 mg/l was unsafe; 0.004 mg/l was safe for several warm- and coldwater fishes, including some salmonids: and the safe level for coho salmon fry (*Oncorhynchus kisutch*) was lower, i.e., between 0.004 mg/l and 0.001 mg/l (McKim and Eaton *unpublished data* 1971).[445]

Daphnia magna appeared to be very sensitive to cadmium. Concentrations of 0.0005 mg/l were found to reduce reproduction in one-generation exposures lasting three weeks (Biesinger and Christensen *unpublished data* 1971).[440] This sensitivity is probably representative of other crustaceans as well.

Recommendation

Aquatic life should be protected where levels of cadmium do not exceed 0.03 mg/l in water having total hardness above 100 mg/l as $CaCO_3$, or 0.004 mg/l in waters with a hardness of 100 mg/l or below at any time or place. Habitats should be safe for crustaceans or the eggs and larvae of salmon if the levels of cadmium do not exceed 0.003 mg/l in hard water or 0.0004 mg/l in soft water at any time or place.

Chromium

The chronic toxicity of hexavalent chromium to fish has been studied by Olson (1958),[422] and Olson and Foster (1956,[423] 1957).[424] Their data demonstrated a pronounced cumulative toxicity of chromium to rainbow trout and chinook salmon (*Oncorhynchus tshawytscha*). Duodoroff and Katz (1953)[406] found that bluegills (*Lepomis macrochirus*) tolerated a 45 mg/l level for 20 days in hard water. Cairns (1956),[403] using chromic oxide (CrO_3), found that a concentration of 104 mg/l was toxic to bluegills in 6 to 84 hours. Bioassays conducted with four species of fish gave 96-hour LC50's of hexavalent chromium that ranged from 17 to 118 mg/l, indicating little effect of hardness on toxicity (Pickering and Henderson 1966).[426]

Recently some tests of chronic effects on reproduction of fish have been carried out. The 96-hour LC50 and safe concentrations for hexavalent chromium were 33 and 1.0 mg/l for fathead minnows (*Pimephales promelas*) in hard water (Pickering *unpublished data* 1971),[446] 50 and 0.6 mg/l for brook trout (*Salvelinus fontinalis*) in soft water, and 69 and 0.3 mg/l for rainbow trout (*Salmo gairdneri*) in soft water (Benoit *unpublished data* 1971).[438] Equivalent values for trivalent chromium were little different: 27 mg/l for the 96-hour LC50, and 1.0 mg/l for a safe concentration for fathead minnows in hard water (Pickering *unpublished data* 1971).[446]

For *Daphnia* the LC50 of hexavalent chromium was reported as 0.05 mg/l, and the chronic no-effect level of trivalent chromium on reproduction was 0.33 mg/l (Biesinger and Christensen *unpublished data* 1971).[440] Some data are available concerning the toxicity of chromium to algae. The concentrations of chromium that inhibited growth for the test organisms are as follows (Hervey 1949):[410] Chlorococcales, 3.2 to 6.4 mg/l; Euglenoids, 0.32 to 1.6 mg/l; and diatoms, 0.032 to 0.32 mg/l. Patrick (*unpublished data* 1971)[447] found that 50 per cent growth reduction for two diatoms in hard and soft water occurred at 0.2 to 0.4 mg/l chromium.

Thus it is apparent that there is a great range of sensitivity to chromium among different species of organisms and in different waters. Those lethal levels reported above are 17 to 118 mg/l for fish, 0.05 mg/l for invertebrates, and 0.032 to 6.4 mg/l for algae, the highest value being 3,700 times the lowest one. The apparent "safe" concentration for fish is moderately high, but the recommended maximum concentration of 0.05 mg/l has been selected in order to protect other organisms, in particular *Daphnia* and certain diatoms which are affected at slightly below this concentration.

Recommendation

Mixed aquatic populations should be protected where the concentration of total chromium in water does not exceed 0.05 mg/l at any time or place.

Copper

Copper is known to be particularly toxic to algae and mollusks, and the implications of this should be considered for any given body of water. Based on studies of effects on these organisms, it is known that the criteria for fish protect these other forms as well. Recent work (Biesinger et al. *unpublished data* 1971)[439] indicated that the safe level of copper for reproduction and growth of *Daphnia magna* in soft water (45 mg/l as $CaCO_3$) is 0.006 mg/l, which is similar to the concentrations described below as safe for fish. The relationship of LC50 to water hardness was shown in Figure III-7 for rainbow trout (*Salmo gairdneri*).

The safe concentration of copper for reproduction by fathead minnows (*Pimephales promelas*) in hard water (200 mg/l as $CaCO_3$) was between 0.015 and 0.033 mg/l (Mount 1968),[418] and in soft water (30 mg/l as $CaCO_3$) was between 0.011 and 0.018 mg/l (Mount and Stephan 1969).[419] More recent work with fathead minnows in hard water indicated that a concentration of 0.033 mg/l would probably be safe (Brungs *unpublished data* 1971).[441] Acceptable reproduction by brook trout (*Salvelinus fontinalis*) in soft water (45 mg/l as $CaCO_3$) occurred between 0.010 and 0.018 mg/l (McKim and Benoit 1971).[416] The safe-to-lethal ratios determined in these studies varied somewhat; but that for hard water is close to 0.1 and that for soft water is approximately 0.1 to 0.2. In very soft water, typical of some northern and mountainous regions, 0.1 of the 96-hour LC50 for sensitive species would be close to what is considered a natural concentration in these waters.

Recent work indicated that avoidance reactions by fish may be as restrictive as reproductive requirements or even more so (Sprague 1964b).[430] It has been demonstrated that Atlantic salmon (*Salmo salar*) avoid a concentration of 0.004 mg/l in the laboratory.

Recommendation

Once a 96-hour LC50 has been determined using the receiving water in question and the most sensitive important species in the locality as the test organism, a concentration of copper safe to aquatic life in that water can be estimated by multiplying the 96-hour LC50 by an application factor of 0.1.

Lead

Lead has a low solubility of 0.5 mg/l in soft water and only 0.003 mg/l in hard water, although higher concentrations of suspended and colloidal lead may remain in the water. The extreme effects of water hardness on lead toxicity are demonstrated by the LC50 values in hard and soft waters. The 96-hour LC50 values in soft water (20 to 45 mg/l as $CaCO_3$) were 5 to 7 mg/l and 4 to 5 mg/l for the fathead minnow (*Pimephales promelas*) and the brook trout (*Salvelinus fontinalis*) respectively (Pickering and Henderson 1966,[426] Benoit *unpublished data* 1971).[438] Brown (1968)[401] reported a 96-hour LC50 of 1 mg/l for rainbow trout (*Salmo gairdneri*) in soft water (50 mg/l as $CaCO_3$). (See Figure III–9 for other values for this species.) The 96-hour LC50 values of lead in hard water were 482 mg/l and 442 mg/l for fathead minnow and brook trout (Pickering and Henderson 1966).[426]

There is not sufficient information on chronic toxicity of lead to fish to justify recommending values as application factors. However, preliminary information on long exposures (2 to 3 months) on rainbow trout and brook trout (Everhart *unpublished data* 1971,[443] Benoit *unpublished data* 1971)[438] indicated detrimental effects at 0.10 mg/l of lead in soft water (20 to 45 mg/l as $CaCO_3$), a safe-to-lethal ratio of less than 0.02.

Growth of guppies (*Lebistes*) was affected by 1.24 mg/l of lead (Crandall and Goodnight 1962).[405] Jones (1939)[411] and Hawksley (1967)[408] found chronic or sublethal effects on sticklebacks from lead concentrations of 0.1 and 0.3 mg/l. The conditioned behavior of goldfish (*Carassius auratus*) in a light-dark shuttlebox was adversely affected by 0.07 mg/l of lead in soft water (Weir and Hine 1970).[437]

Chronic lead toxicity was recently investigated with *Daphnia magna* (Biesinger and Christensen *unpublished data* 1971)[440] and the effect on reproduction was observed at a level of 0.03 mg/l of lead. This concentration of 0.03 mg/l, the safe level for *Daphnia*, is recommended as the criterion for protection of aquatic life. It is probably also close to the safe level for fish, because the tests described above, although somewhat preliminary, indicated that concentrations about 2 or 3 times higher had detrimental effects.

Recommendation

The concentration of lead in water should not be higher than 0.03 mg/l at any time or place in order to protect aquatic life.

Mercury

Most data about mercury involve the organic compounds (see the discussion of Organic Mercury, p. 172.) Information is available, however, for inorganic mercury in the form of mercuric ions. Short-term 96-hour bioassay studies indicated that concentrations of 1 mg/l are fatal to fish (Boetius 1960,[400] Jones 1939,[411] Weir and Hine 1970).[437] For long-term exposures of 10 days or more, mercury levels as low as 10 to 20 mg/l have been shown to be fatal to fish (Uspenskaya 1946).[436]

Recommendation

In protecting aquatic life, the recommendations for organic mercury (p. 174) also pertain here.

Nickel

The 96-hour LC50 of nickel for fathead minnows (*Pimephales promelas*) ranges from 5 mg/l in soft water (20 mg/l as $CaCO_3$) to 43 mg/l in hard water (360 mg/l as $CaCO_3$) under static test conditions (Pickering and Henderson 1966).[426] In water of 200 mg/l hardness (as $CaCO_3$), the 96-hour LC50 for fathead minnows was 26 to 31 mg/l with a chronically safe concentration between 0.8 and 0.4 mg/l (Pickering *unpublished data* 1971).[446] On the basis of this work, an application factor of 0.02 appeared to be appropriate for the protection of fish. If this factor is used, the estimated safe concentration of nickel for fathead minnows in soft water would be about 0.1 mg/l. Using static test conditions and *Daphnia magna*, Biesinger and Christensen (*unpublished data* 1971)[440] determined that a nickel concentration of 0.095 mg/l reduced reproduction during a 3-week exposure in soft water (45 mg/l as $CaCO_3$), and a nickel concentration of 0.030 mg/l had no effect. This result indicated that the sensitivity of *Daphnia magna* is comparable to that of fish.

Recommendation

Once a 96-hour LC50 has been determined using the receiving water in question and the most sensitive important species in the locality as the test organism, a concentration of nickel safe to aquatic

life in that water can be estimated by multiplying the 96-hour LC50 by an application factor of 0.02.

Zinc

The acute lethal toxicity of zinc is greatly affected by water hardness (see Figure III–7). Pickering and Henderson (1966)[425] determined the 96-hour LC50 of zinc for fathead minnows (*Pimephales promelas*) and bluegills (*Lepomis macrochirus*) using static test conditions. For fathead minnows in soft water (20 mg/l as $CaCO_3$) the LC50 was 0.87 mg/l, and in hard water (360 mg/l as $CaCO_3$) it was 33 mg/l. Bluegills were more resistant in both waters. Similarly the lethal threshold concentration was 3 or 4 times as high for coarse fish as for trout (*Salvelinus fontinalis*) (Ball 1967).[388]

The 24-hour LC50 of zinc for rainbow trout (*Salmo gairdneri*) was reduced only 20 per cent when the fish were forced to swim at 85 per cent of their maximum sustained swimming speed (Herbert and Shurben 1964).[409] The maximum effect of a reduction in dissolved oxygen from 6 to 7 mg/l to 2 mg/l on the acute toxicity of zinc was a 50 per cent increase (Lloyd 1961,[413] Cairns and Scheier 1958,[404] Pickering 1968).[425] The effects are small in comparison to the difference between acutely toxic and safe concentrations. The recommended application factor recognizes these effects.

A chronic test in hard water (200 mg/l as $CaCO_3$), involving fathead minnow reproduction, determined the safe concentration of zinc to be between 0.03 mg/l, which had no effect, and 0.18 mg/l, which caused 83 per cent reduction in fecundity (Brungs 1969).[402] Using the 96-hour LC50 of 9.2 mg/l, the ratio of the above no-effect concentration to the LC50 is 0.0034. Interpolation suggests that about 0.005 of the LC50 would cause 20 per cent reduction of fecundity, making the best estimate of a valid application factor close to 0.005.

There was a reduction in reproduction of *Daphnia magna* at a zinc concentration of 0.10 mg/l using soft water (45 mg/l as $CaCO_3$) (Biesinger and Christensen *unpublished data* 1971).[449] No effect was observed at 0.07 mg/l, which indicated that *Daphnia magna* was more resistant to zinc than the fathead minnow.

Avoidance reactions by rainbow trout in the laboratory have been caused by 0.01 of the LC50 of zinc (Sprague 1968a).[431]

Recommendation

Once a 96-hour LC50 has been determined using the receiving water in question and the most sensitive important species in the locality as the test organism, a concentration of zinc safe to aquatic life in that water can be estimated by multiplying the 96-hour LC50 by an application factor of 0.005.

PESTICIDES

Pesticides are chemicals, natural and synthetic, used to control or destroy plant and animal life considered adverse to human society. Since the 1940's a large number of synthetic organic compounds have been developed for pesticide purposes. Presently there are thousands of registered formulations incorporating nearly 900 different chemicals. Trends in production and use of pesticides indicate an annual increase of about 15 per cent, and there are predictions of increased demand during the next decade (Mrak 1969).[477] The subject of pesticides and their environmental significance has been carefully evaluated in the Report of the Secretary's Commission on Pesticides and their Relationship to Environmental Health (Mrak 1969).[477]

Methods, Rate, and Frequency of Application

Pesticides are used for a wide variety of purposes in a multitude of environmental situations. Often they are categorized according to their use or intended target (e.g., insecticide, herbicide, fungicide), but their release in the environment presents an inherent hazard to many non-target organisms. Some degree of contamination and risk is assumed with nearly all pesticide use. The risk to aquatic ecosystems depends upon the chemical and physical properties of the pesticide, type of formulation, frequency, rate and methods of application, and the nature of the receiving system.

The pesticides of greatest concern are those that are persistent for long periods and accumulate in the environment; those that are highly toxic to man, fish, and wildlife; and those that are used in large volumes over broad areas. A list of such chemicals recommended for monitoring in the environment appears in Appendix II–F. The majority of these compounds are either insecticides or herbicides used extensively in agriculture, public health, and for household or garden purposes. In the absence of definitive data on their individual behavior and their individual effect on the environment, some generalization about pesticides is required to serve as a guideline for establishing water quality criteria to protect aquatic life. In specific instances, however, each compound must be considered individually on the basis of information about its reaction in the environment and its effect on aquatic organisms.

Sources and Distribution

The major sources of pesticides in water are runoff from treated lands, industrial discharges, and domestic sewage. Significant contributions may also occur in fallout from atmospheric drift and in precipitation (Tarrant and Tatton 1968).[488] Applications to water surfaces, intentional or otherwise, will result in rapid and extensive contamination. The persistent organochlorine pesticides have received the greatest attention in monitoring programs (Lichtenberg et al. 1970,[471] Henderson et al. 1969).[461] Their extensive distribution in aquatic systems is indicative of environmental loading from both point and nonpoint sources.

Many pesticides have a low water solubility that favors their rapid sorption on suspended or sedimented materials and their affinity to plant and animal lipids. Soluble or dispersed fractions of pesticides in the water rapidly decline after initial contamination. resulting in increased concentrations in the sediments (Yule and Tomlin 1971).[489] In streams, much of the residue is in continuous transport on suspended particulate material or in sediments (Zabik 1969).[490] The distribution within the stream flow is nonuniform because of unequal velocity and unequal distribution of suspended materials within the stream bed (Feltz et al. 1971).[454] Seasonal fluctuations in runoff and use pattern cause major changes in concentration during the year, but the continuous downstream transport tends to reduce levels in the upper reaches of streams while increasing them in the downstream areas and eventually in major receiving basins (i.e., lakes, reservoirs, or estuaries). If applications in a watershed cease entirely, residues in the stream will gradually and continuously decline (Sprague et al. 1971).[484] A similar decline would be expected in the receiving basins but at a slower rate.

In lakes the sediments apparently act as a reservoir from which the pesticide is partitioned into the water phase according to the solubility of the compound, the concentration in the sediment, and the type of sediment (Hamelink et al. 1971).[458] Dissolved natural organic materials in the water may greatly enhance the water solubility of some pesticides (Wershaw et al. 1969).[487] Some investigations indicated pesticides may be less available to the water in eutrophic systems where the higher organic content in the sediments has a greater capacity to hold pesticide residues (Lotse et al. 1968,[472] Hartung 1970[460]). This in part explained the difference in time required for some waters to "detoxify," as observed in lakes treated with toxaphene to eradicate undesirable fish species (Terriere et al. 1966).[486]

Herbicides applied to aquatic systems to control plant growths are removed from the water by absorption in the plants or sorption to the hydrosoil. The rate of disappearance from the water may be dependent upon the availability of suitable sorption sites. Frank and Comes (1967)[455] found residues of dichlobenil in soil and water up to 160 days after application. They also found that diquat and paraquat residues were persistent in hydrosoils for approximately 3 to 6 months after application. Granular herbicide treatments made on a volume basis deposit greater quantities on the hydrosoil in deep water areas than in water of less depth. The granules may supply herbicide to the water over a period of time depending upon solubility of the herbicide, concentrations in the granule, and other conditions.

Because the distribution of pesticides is nonuniform, sampling methods and frequency, as well as selection of sampling sites, must be scientifically determined (Feltz et al. 1971).[454] Pesticides found in the water in suspended particulate material and in sediments may be toxic to aquatic organisms or contribute to residue accumulation in them.

Persistence and Biological Accumulation

All organic pesticides are subject to metabolic and nonmetabolic degradation in the environment. Specific compounds vary widely in their rate of degradation, and some form degradation products that may be both persistent and toxic. Most pesticides are readily degraded to nontoxic or elementary materials within a few days to a few months; these compounds may be absorbed by aquatic organisms, but the residues do not necessarily accumulate or persist for long periods. Concentrations in the organism may be higher than ambient water levels, but they rapidly decline as water concentrations are diminished. Examples of such dynamic exchange have been demonstrated with malathion (Bender 1969),[448] methoxychlor (Burdick et al. 1968),[449] and various herbicides (Mullison 1970).[478] If degradation in water is completed within sufficient time to prevent toxic or adverse physiological effects, these nonpersistent compounds do not pose a long-term hazard to aquatic life. However, degradation rates of specific pesticides are often dependent upon environmental conditions. Considerable variation in persistence may be observed in waters of different types. Gakstatter and Weiss (1965),[456] for example, have shown that wide variations in the stability of organic phosphorous insecticides in water solutions is dependent upon the pH of the water. The half-life of malathion was reduced from about six months at pH 6 to only one to two weeks at pH 8. Repeated applications and slow degradation rates may maintain elevated environmental concentrations, but there is no indication that these compounds can be accumulated through the food chain.

Some pesticides, primarily the organochlorine compounds, are extremely stable, degrading only slowly or forming persistent degradation products. Aquatic organisms may accumulate these compounds directly by absorption from water and by eating contaminated food organisms. In waters containing very low concentrations of pesticides, fish probably obtain the greatest amount of residue from contaminated foods; but the amount retained in the tissue appears to be a function of the pesticide concentration in the water and its rate of elimination from the organism (Hamelink et al. 1971).[458] The transfer of residues from prey to predator in the food chain ultimately results in residues in the higher trophic levels many thousand times higher than ambient water levels. Examples of trophic accumulation have been described in several locations including Clear Lake, California (Hunt and Bischoff 1960),[463] and Lake Poinsett, South Dakota (Hannon et al. 1970).[459]

Residues

Samples of wild fish have often contained pesticide residues in greater concentrations than are tolerated in any commercially produced agricultural products. The highest concentrations are often found in the most highly prized fish. Coho salmon (*Oncorhyncus kisutch*) from Lake Michigan are not considered acceptable for sale in interstate commerce on the basis of an interim guideline for DDT and its metabolites set for fish by the U.S. Food and Drug Administration (Mount 1968).[476] Lake trout (*Salvelinus namaycush*) and some catches of chubs (*Coregonus kiyi* and *Coregonus hoyi*) and lake herring (*Coregonus artedi*) from Lake Michigan also exceed the guideline limits and are thus not considered acceptable for interstate commerce (Reinert 1970;[481] Michigan Department of Agriculture *personal communication*).[492] Pesticide residues in fish or fish products may enter the human food chain indirectly in other ways, as in fish oil and meal used in domestic animal feeds.

Fish may survive relatively high residue concentrations in their body fats, but residues concentrated in the eggs of mature fish may be lethal to the developing fry. Up to 100 per cent loss of lake trout (*Salvelinus namaycush*) fry occurred when residues of DDT–DDD in the eggs exceeded 4.75 mg/kg (Burdick et al. 1964).[450] A similar mortality was reported in coho salmon fry from Lake Michigan where eggs contained significant quantities of DDT, dieldrin, and polychlorinated biphenyls (Johnson and Pecor 1969;[468] Johnson 1968).[466] Johnson (1967)[467] reported that adult fish not harmed by low concentrations of endrin in water accumulated levels in the eggs that were lethal to the developing fry. Residues in fish may be directly harmful under stress conditions or at different temperature regimes. Brook trout (*Salvelinus fontinalis*) fed DDT at 3.0 mg/kg body weight per week for 26 weeks suffered 96.2 per cent mortality during a period of reduced feeding and declining water temperature. Mortality of untreated control fish during the same period was 1.2 per cent (Macek 1968).[473] Declining water temperature during the fall was believed to cause delayed mortality of salmon parr in streams contaminated with DDT (Elson 1967).[453]

In addition to the problem of pesticide residues in aquatic systems, other problems suggest themselves and remain to be investigated, including the potential of resistant fish species to accumulate levels hazardous to other species (Rosato and Ferguson 1968);[482] the potential for enhanced residue storage when fish are exposed to more than one compound (Mayer et al. 1970);[474] and the potential effect of metabolites not presently identified. The adverse effects of DDT on the reproductive performance of fish-eating birds has been well documented. (See the discussion of Wildlife, pp. 194–198.)

Levels of persistent pesticides in water that will not result in undesirable effects cannot be determined on the basis of present knowledge. Water concentrations below the practical limits of detection have resulted in unacceptable residues in fish for human consumption and have affected reproduction and survival of aquatic life. Criteria based upon residue concentrations in the tissues of selected species may offer some guidance. Tolerance levels for pesticides in wild fish have not been established, but action levels have been suggested by the U.S. Food and Drug Administration (Mount 1968).[476] However, acceptable concentrations of persistent pesticides that offer protection to aquatic life and human health are unknown.

It should also be recognized that residue criteria are probably unacceptable except on a total ecosystem basis. Residues in stream fish may meet some guidelines, but pesticides from that stream may eventually create excessive residues in fish in the downstream receiving basins. Until more is known of the effects of persistent pesticide residues, any accumulation must be considered undesirable.

Toxicity

Concentrations of pesticides that are lethal to aquatic life have often occurred in local areas where applications overlap streams or lakes, in streams receiving runoff from recently treated areas, and where misuse or spillage has occurred. Applications of pesticides to water to control noxious plants, fish, or insects have also killed desirable species. Fish populations, however, usually recovered within a few months to a year (Elson 1967).[453] The recovery of aquatic invertebrates in areas that have been heavily contaminated may require a longer period, with some species requiring several years to regain precontamination numbers (Cope 1961,[451] Ide 1967).[465] Undesirable species of insects may be the first to repopulate the area (Hynes 1961),[464] and in some instances the species composition has been completely changed (Hopkins et al. 1966).[462] Areas that are contaminated by pesticide application are subject to loss of fish populations and reduced food for fish growth (Schoenthal 1964,[483] Kerswill and Edwards 1967).[469] Where residues are persistent in bottom sediments for long periods, benthic organisms may be damaged even though water concentrations remain low (Wilson and Bond 1969).[488]

Pesticides are toxic to aquatic life over wide ranges. Great differences in susceptibility to different compounds exist between species and within species. For example, 96-hour LC50 values of 5 to 610,000 μg/l were reported for various fish species exposed to organophosphate pesticides (Pickering et al. 1962).[479] In addition to species' differences, the toxicity may be modified by differences in formulation, environmental conditions, animal size and age, and physiological condition. The effect of combinations of pesticides on aquatic organisms has not received sufficient attention. Macek (*unpublished data* 1971)[491] reported that combinations of various common pesticides were synergistic in their action on bluegill (*Lepomis macrochirus*) and rainbow trout (*Salmo gairdneri*), while others had additive effects. Several of the combinations that were found to be synergistic are recommended for insect pest control (Table III–16).

TABLE III-16—Acute Toxic Interaction of Pesticide Combinations to Rainbow Trout and Bluegills.

Pesticide combination		
Compound A	Compound B	Toxic interaction
DDT	Vapona	Additive
"	Endrin	Additive
"	Dieldrin	Additive
"	Azinphosmethyl	Additive
"	Toxaphene	Additive[a]
"	Zectran	Additive
"	BHC	Synergistic[a]
Parathion	Copper sulfate	Synergistic
"	Diazinon	Synergistic
"	DDT	Additive[a]
"	Endosulfan	Additive
"	Methoxychlor	Synergistic[a]
Malathion	Baytex	Synergistic
"	Copper sulfate	Antagonistic
"	DDT	Additive
"	EPN	Synergistic
"	Parathion	Synergistic
"	Perthane	Synergistic[a]
"	Carbaryl	Synergistic[a]
"	Toxaphene	Additive[a]
Carbaryl	Copper sulfate	Synergistic
"	DDT	Additive
"	Azinphosmethyl	Additive[a]
"	Methoxychlor	Additive
"	Parathion	Additive[a]
Methyl parathion	DDT	Additive
"	Endosulfan	Additive[a]
"	Carbaryl	Additive[a]
Bidrin	Sumithion	Additive

[a] This combination recommended for control of insect pests by the U.S. Department of Agriculture.

Note: mention of trade names does not constitute endorsement.

Most data on pesticide effects on aquatic life are limited to a few species and concentrations that are lethal in short-term tests. The few chronic tests conducted with aquatic species indicated that toxic effects occurred at much lower concentrations. Mount and Stephan (1967)[476] found the 96-hour LC50 for fathead minnows (*Pimephales promelas*) in malathion was 9,000 μg/l, but spinal deformities in adult fish occurred during a 10-month exposure to 580 μg/l. Eaton (1970)[452] found that bluegill suffered the same crippling effects after chronic exposure to 7.4 μg/l malathion and the 96-hour LC50 was 108 μg/l.

Where chronic toxicity data are available, they may be used to develop application factors to estimate safe levels. Mount and Stephan (1967)[476] have suggested using an application factor consisting of the laboratory-determined maximum concentration that has no effect on chronic exposure divided by the 96-hour LC50. Using this method, Eaton (1970)[452] showed that application factors for bluegill and fathead minnow exposed to malathion were similar despite a greater than 50-fold difference in species sensitivity. Application factors derived for one compound may be appropriate for closely related compounds that have a similar mode of action, but additional research is necessary to verify this concept. In the absence of chronic toxicity data, the application factors for many compounds must be arbitrary values set with the intention of providing some margin of safety for sensitive species, prolonged exposure, and potential effects of interaction with other compounds.

Basis for Criteria

The reported acute toxicity values and subacute effects of pesticides for aquatic life are listed in Appendix II-D. The acute toxicity values multiplied by the appropriate application factor provided the recommended criteria. The 96-hour LC50 should be multiplied by an application factor of 0.01 in most cases. The value derived from multiplying the 96-hour LC50 by a factor of 0.01 can be used as the 24-hour average concentration.

Recommended concentrations of pesticides may be below those presently detectable without additional extraction and concentration techniques. However, concentrations below those detectable by routine techniques are known to cause detrimental effects to aquatic organisms and to man. Therefore, recommendations are based on bioassay procedures and the use of an appropriate application factor.

The recommendations are based upon the most sensitive species. Permissible concentrations in water have been suggested only where several animal species have been tested. Where toxicity data are not available, acute toxicity bioassays should be conducted with locally important sensitive aquatic species, and safe levels should be estimated by using an application factor of 0.01.

Some organochlorine pesticides (i.e., DDT including DDD and DDE, aldrin, dieldrin, endrin, chlordane, heptachlor, toxaphene, lindane, endosulfan, and benzene hexachloride) are considered especially hazardous because of their persistence and accumulation in aquatic organisms. These compounds, including some of their metabolites, are directly toxic to various aquatic species at concentrations of less than one μg/l. Their accumulation in aquatic systems presents a hazard, both real and potential, to animals in the higher trophic levels, including man (Pimentel 1971,[450] Mrak 1969,[477] Kraybill 1969,[470] Gillett 1969).[457] Present knowledge is not yet sufficient to predict or estimate safe concentrations of these compounds in aquatic systems. However, residue concentrations in aquatic organisms provide a measure of environmental contamination. Therefore, specific maximum tissue concentrations have been recommended as a guideline for water quality control.

Recommendations

Organochlorine Insecticides **The recommendations for selected organochlorine insecticides are based upon levels in water and residue concentrations in whole fish on a wet weight basis. Aquatic life should be protected where the maximum con-**

centration of the organochlorine pesticide in the water does not exceed the values listed in Table III-17.

For the protection of predators, the following values are suggested for residues in whole fish (wet weight): DDT (including DDD and DDE)—1.0 mg/kg; aldrin, dieldrin, endrin, heptachlor (including heptachlor epoxide), chlordane, lindane, benzene hexachloride, toxaphene, and endosulfan—0.1 mg/kg, either singly or in combination. For further discussion, see the section on Wildlife (p. 197).

If fish and wildlife are to be protected, and where residues exceed the recommended concentrations, pesticide use should be restricted until the recommended concentrations are reached (except where a substitute pesticide will not protect human health).

Other pesticides The recommended maximum concentrations of pesticides in freshwater are listed in Table III-18 except that where pesticides are applied to water to kill undesirable aquatic life, the values will be higher. In the latter instances, care should be taken to avoid indiscriminate use and to insure that application of the pesticide follows the prescribed methods.

OTHER TOXICANTS

Ammonia

Ammonia is discharged from a wide variety of industrial processes and cleaning operations that use ammonia or ammonia salts. Ammonia also results from the decomposition of organic matter.

Ammonia gas is soluble in water in the form of ammonium hydroxide to the extent of 100,000 mg/l at 20 C. Ammonium hydroxide dissociates readily into ammonium

TABLE III-17—Recommended Maximum Concentrations of Organochlorine Pesticides in Whole (Unfiltered) Water, Sampled at Any Time and Any Place.[a]

Organochlorine pesticides	Recommended maximum concentration (μg/l)
Aldrin	0.01
DDT	0.002
TDE	0.006
Dieldrin	0.005
Chlordane	0.04
Endosulfan	0.003
Endrin	0.002
Heptachlor	0.01
Lindane	0.02
Methoxychlor	0.005
Toxaphene	0.01

[a] Concentrations were determined by multiplying the acute toxicity values for the more sensitive species (Appendix II-D) by an application factor of 0.01 except where an experimentally derived application factor is indicated.

TABLE III-18—Recommended Maximum Concentrations of Other Pesticides in Whole (Unfiltered) Water, Sampled at Any Time and Any Place.[a]

Organophosphate insecticides	Recommended maximum concentration (μg/l)
Abate	(b)
Azinphosmethyl	0.001
Azinphosethyl	(b)
Carbophenothion	(b)
Chlorothion	(b)
Ciodrin	0.1
Coumaphos	0.001
Demeton	(b)
Diazinon	0.009
Dichlorvos	0.001
Dioxathion	0.09
Disulfoton	0.05
Dursban	0.001
Ethion	0.02
EPN	0.06
Fenthion	0.006
Malathion	0.008
Methyl Parathion	(b)
Mevinphos	0.002
Naled	0.004
Oxydemeton methyl	0.4
Parathion	0.0004
Phorate	(b)
Phosphamidon	0.03
Ronnel	(b)
TEPP	0.4
Trichlorophon	0.002
Carbamate insecticides	**Recommended maximum concentrations (μg/l)**
Aminocarb	(b)
Bayer	(b)
Baygon	(b)
Carbaryl	0.02
Zectran	0.1
Herbicides, fungicides and defoliants	**Recommended maximum concentrations (μg/l)**
Acrolein	(b)
Aminotriazole	300.0
Balan	(b)
Bensulide	(b)
Chloroxuron	(b)
CIPC	(b)
Dacthal	(b)
Dalapon	110.0
DEF	(b)
Dexon	(b)
Dicamba	200
Dichlobenil	37.0
Dichlone	0.2
Diquat	0.5
Diuron	1.6
Difolitan	(b)
Dinitrobutyl phenol	(b)
Diphenamid	(b)
2,4-D (PGBE)	(b)
2,4-D (BEE)	4.0
2,4-D (IOE)	(b)
2,4-D (Diethylamine salts)	(b)
Endothal (Disodium salt)	(b)
Endothal (Dipotassium salt)	(b)
Eptam	(b)
Fenac (Sodium salt)	45.0
Hyamine-1622	(b)
Hyamine-2389	(b)
Hydrothol-47	(b)
Hydrothol-191	(b)
Hydrothol plus	(b)
IPC	(b)
MC[illegible]	(b)
Molinate	(b)
Monuron	(b)
Paraquat	(b)

TABLE III-18—Continued

Herbicides, fungicide and defoliants	Recommended maximum concentration (µg/l)
Pebulate	(b)
Picloram	(b)
Propanil	(b)
Silvex (BEE)	2.5
Silvex (PGBE)	2.0
Silvex (IOE)	(b)
Silvex (Potassium salt)	(b)
Simazine	10.0
Trifluralin	(b)
Vernolate	(b)
Botanicals	**Recommended maximum concentrations (µg/l)**
Allethrin	0.002
Pyrethrum	0.01
Rotenone	10.0

[a] Concentrations were determined by multiplying the acute toxicity values for the more sensitive species (Appendix II-D) by an application factor of 0.01 except where an experimentally derived application factor is indicated.

[b] Insufficient data to determine safe concentrations.

TABLE III-19—Sublethal and Acutely Toxic Concentrations of Un-Ionized Ammonia for Various Fish Species[a]

Species	Acute mortality LC50 (mg/l)	No sublethal effect (mg/l)	Author
Stickleback	1.0-2.1		Hazel et al. (1971)[500]
Striped bass (Morone saxatilis)	1.9-2.8		"
Rainbow trout	0.35	0.046	Lloyd and Orr (1969)[503]
Perch (Perca)	0.29		Ball (1967)[492]
Roach (Hesperoleucus)	0.35		"
Rudd (Scardinius)	0.36		"
Bream (Lepomis)	0.41		"
Rainbow trout	0.41		"
Rainbow trout	0.42-0.89		Lloyd and Herbert (1960)[504]
Atlantic salmon (Salmo salar)	0.38		Herbert and Shurben (1965)[501]
Rainbow trout	0.08		"
Trout		<0.27	Reichenbach-Klinke (1967)[506]
Chinook salmon (Oncorhynchus tshawytscha)		<0.006	Burrows (1964)[495]

[a] To insure a high level of protection, the mean of the 96-hour LC50's was used as a base, and an application factor of 0.05 applied to arrive at an acceptable level for most species in fresh water. Two apparently resistant species were omitted because they were far out of line with the others. After application of the factor, the resultant level is approximately half that projected from the data of Lloyd and Orr (1969).[503]

and hydroxyl ions as follows:

$$NH_3+H_2O \rightarrow HN_4^+ + OH^-$$

The equilibrium of the reaction is dependent upon pH, and within the pH range of most natural waters ammonium ions predominate (Figure III-10). Since the toxic component of ammonia solutions is the un-ionized ammonia, toxicity of ammonia solutions increases with increased pH (Ellis 1937,[497] Wuhrmann et al. 1947,[508] Wuhrmann and Woker 1948,[509] Downing and Merkens 1955[496]).

Wuhrmann (1952),[507] Downing and Merkens (1955),[496] and Merkens and Downing (1957)[505] found that a decrease in dissolved oxygen concentration increased the toxicity of un-ionized ammonia to several species of freshwater fishes. Lloyd (1961)[502] showed that the increase in toxicity of un-ionized ammonia to rainbow trout (*Salmo gairdneri*) with decreased oxygen was considerably more severe than for zinc, copper, lead, or phenol.

Much of the data on ammonia toxicity is not useable, because reporting of chemical conditions or experimental control was unsatisfactory. Ellis (1937)[497] reported that total ammonia nitrogen concentrations of 2.5 mg/l in the pH range of 7.4 to 8.5 were harmful to several fish species, but concentrations of 1.5 mg/l were not. Most streams without a source of pollution contained considerably less than 1 mg/l total ammonia. The sublethal and acutely toxic concentrations of un-ionized ammonia for various fish species are given in Table III-19.

Brockway (1950)[494] found impairment of oxygen-carrying capacity of the blood of trout at a total ammonia nitrogen concentration of 0.3 mg/l. Fromm (1970)[499] found that at total ammonia nitrogen concentrations of 5 mg/l, ammonia excretion by rainbow trout (*Salmo gairdneri*) was inhibited; at 3 mg/l the trout became hyperexcitable; and at 8 mg/l (approximately 1 mg/l un-ionized ammonia) 50 per cent were dead in 24 hours (Fromm 1970).[499] Goldfish (*Carassius auratus*) were more tolerant; at 40 mg/l of total ammonia nitrogen, 10 per cent were dead in 24 hours.

Burrows (1964)[495] found progressive gill hyperplasia in fingerling chinook salmon (*Oncorhynchus tshawytscha*) during a six-week exposure to the lowest concentration applied, 0.006 mg/l un-ionized ammonia. Reichenbach-Klinke (1967)[506] also noted gill hyperplasia, as well as pathology of the liver and blood, of various species at un-ionized ammonia concentrations of 0.27 mg/l. Exposure of carp (*Cyprinus carpio*) to sublethal un-ionized ammonia concentrations in the range of 0.11 to 0.34 mg/l resulted in extensive necrotic changes and tissue disintegration in various organs (Flis 1968).[498]

Lloyd and Orr (1969)[503] found that volume of urine production increased with exposure to increasing ammonia concentrations, but that an ammonia concentration of 12 per cent of the lethal threshold concentration resulted in no increased production of urine. This concentration of un-ionized ammonia was 0.046 mg/l for the rainbow trout used in the experiments.

Recommendation

Once a 96-hour LC50 has been determined using the receiving water in question and the most sensitive important species in the locality as the test organism, a concentration of un-ionized ammonia (NH_3) safe to aquatic life in that water can be estimated by multiplying the 96-hr LC50 by an application factor of 0.05, but no concentration greater than 0.02 mg/l is recommended at any time or place.

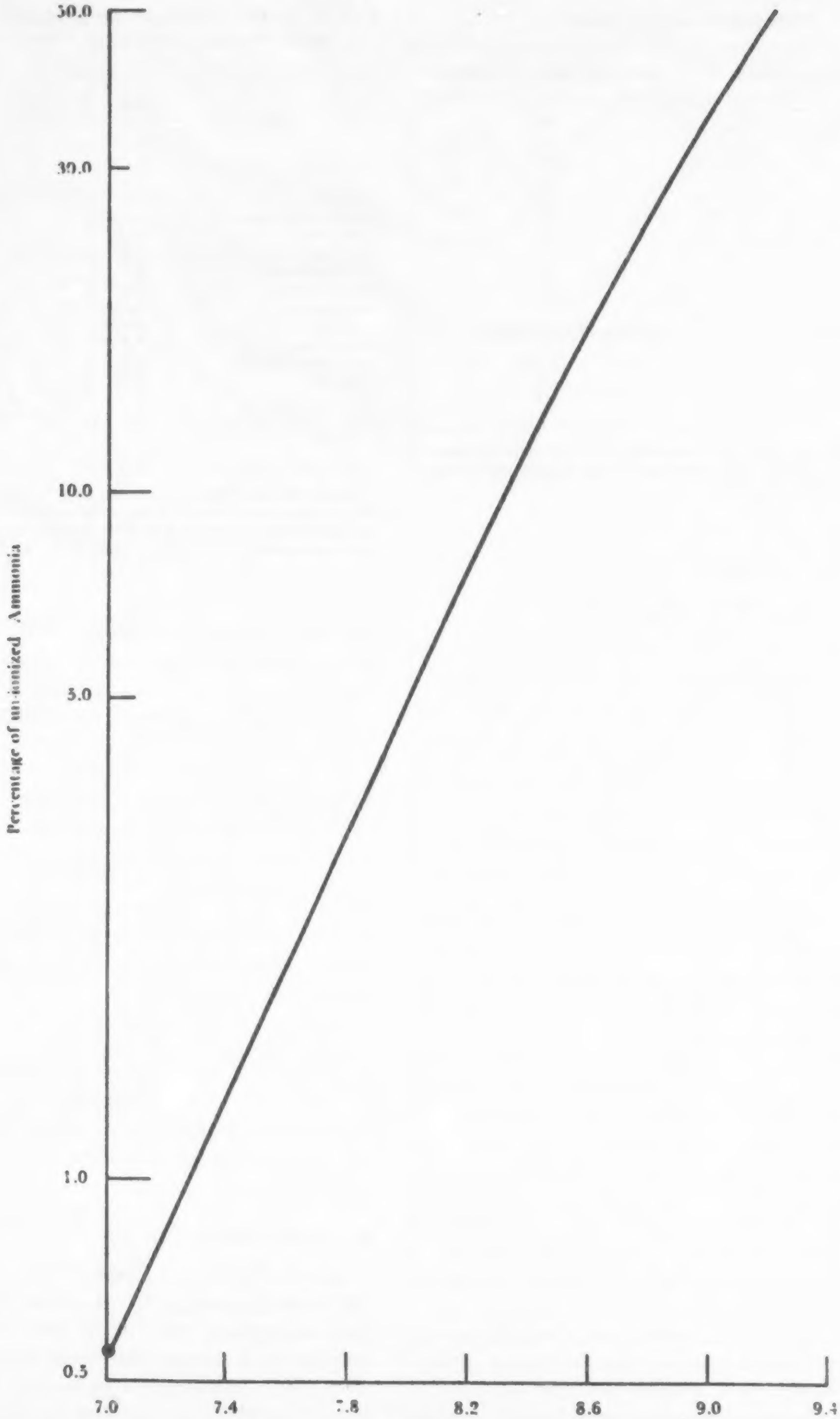


FIGURE III-10—Percentage of Un-ionized Ammonia in Ammonium Hydroxide Solutions at 20 C and Various Levels of pH

Chlorine

Chlorine and chloramines are widely used in treatment of potable water supplies and sewage-treatment-plant effluents, and in power plants, textile and paper mills, and certain other industries. Field tests conducted on caged fish in streams below a sewage outfall where chlorinated and non-chlorinated effluents were discharged showed that toxic conditions occurred for rainbow trout (*Salmo gairdneri*) 0.8 miles below the plant discharge point when chlorinated effluents were discharged (Basch et al. 1971).[511] It has also been shown that total numbers of fish and numbers of species were drastically reduced below industrial plants discharging chlorinated sewage effluents (Tsai 1968,[517] 1970).[518]

The toxicity to aquatic life of chlorine in water will depend upon the concentration of residual chlorine remaining and the relative amounts of free chlorine and chloramines. Since addition of chlorine or hypochlorites to water containing nitrogenous materials rapidly forms chloramines, problems of toxicity in most receiving waters are related to chloramine concentrations. Merkens (1958)[515] stated that toxicities of free chlorine and chloramines were best estimated from total chlorine residuals. In monitoring programs, evaluation of chlorine content of water is usually stated in terms of total chlorine residuals. Because the chlorine concentrations of concern are below the level of detection by the orthotolidine method, a more sensitive analytical technique is recommended.

The literature summarized by McKee and Wolf (1963)[514] showed a wide range of acute chlorine toxicity to various aquatic organisms, but the conditions of the tests varied so widely that estimation of generally applicable acute or safe levels cannot be derived from the combined data. It has also been demonstrated that small amounts of chlorine can greatly increase the toxicity of various industrial effluents.

Merkens (1958)[515] found that at pH 7.0, 0.008 mg/l residual chlorine killed half the test fish in seven days. The test results were obtained using the amperometric titration and the diethyl-*p*-phenylene diamine methods of chlorine analysis. Zillich (1972),[519] working with chlorinated sewage effluent, determined that threshold toxicity for fathead minnows (*Pimephales promelas*) was 0.04–0.05 mg/l residual chlorine. In two series of 96-hour LC50 tests an average of 0.05–0.19 mg/l residual chlorine was noted. Basch et al. (1971)[511] found 96-hour LC50 for rainbow trout (*Salmo gairdneri*) to be 0.23 mg/l. Arthur and Eaton (1971),[510] working with fathead minnows and *Gammarus pseudolimnaeus*, found that the 96-hour LC50 total residual chlorine (as chloramine) for *Gammarus* was 0.22 mg/l, and that all minnows were dead after 72 hours at 0.15 mg/l. After seven days exposure to 0.09 mg/l, the first fish died. The LC50 for minnows was therefore between these levels. In chronic tests extending for 15 weeks, survival of *Gammarus* was reduced at 0.04 mg/l, and reproduction was reduced at 0.0034 mg/l. Growth and survival of fathead minnows after 21 weeks was not affected by continuous exposure to 0.043 mg/l total chloramines, but fecundity of females was reduced. The highest level showing no significant effect was 0.016 mg/l. Merkens (1958)[515] postulated by extrapolation that a concentration of 0.004 mg/l residual chlorine would permit one half the test fish to survive one year. Sprague and Drury (1969)[516] have shown an avoidance response of rainbow trout to free chlorine at 0.001 mg/l.

Aquatic organisms will tolerate longer short-term exposures to much higher levels of chlorine than the concentrations which have adverse chronic effects. Brungs (1972)[512] in a review has noted that 1-hour LC50's of fish vary from 0.74 to 0.88 mg/l, and that longer short-term exposures have LC50's lower but still substantially higher than acceptable for long-term exposure. Available information, however, does not show what effect repeated exposure to these, or lower levels, will have on aquatic life.

Because *Gammarus*, an essential food for fish, is affected at 0.0034 mg/l, and a safe level is judged to be one that will not permit adverse effect on any element of the biota, the following recommendation has been made.

Recommendation

Aquatic life should be protected where the concentration of residual chlorine in the receiving system does not exceed 0.003 mg/l at any time or place. Aquatic organisms will tolerate short-term exposure to high levels of chlorine. Until more is known about the short-term effects, it is recommended that total residual chlorine should not exceed 0.05 mg/l for a period up to 30-minutes in any 24-hour period.

Cyanides

The cyanide radical is a constituent of many compounds or complex ions that may be present in industrial wastes. Cyanide-bearing wastes may derive from gas works, coke ovens, scrubbing of gases in steel plants, metal plating operations, and chemical industries. The toxicity of cyanides varies widely with pH, temperature, and dissolved oxygen concentration. The pH is especially important, since the toxicity of some cyanide complexes changes manyfold over the range commonly found in receiving waters.

"Free cyanide" (CN^- ion and HCN) occurs mostly as molecular hydrogen cyanide, the more toxic form, at pH levels of natural waters as well as in unusually acid waters. Fifty per cent ionization of the acid occurs at pH near 9.3. Free cyanide concentrations from [illegible] 0.01 mg/l as CN have proved fatal to many sensitive fishes (Jones 1964),[527] and levels much above 0.[illegible] rapidly fatal for most species of fish. A level [illegible] mg/l is known to have a pronounced, rapid, and [illegible] effect on the swimming ability of salmonid fishes.

The work of Doudoroff et al. (1966)[524] has demonstrated that the effective toxicant to fish in nearly all solutions of complex metallocyanides tested was molecular HCN, the complex ions being relatively harmless. The total cyanide content of such solutions is not a reliable index of their toxicity. The HCN derives from dissociation of the complex ions, which can be greatly influenced by pH changes. Doudoroff (1956)[523] demonstrated a more than thousand-fold increase of the toxicity of the nickelocyanide complex associated with a decrease of pH from 8.0 to 6.5. A change in pH from 7.8 to 7.5 increased the toxicity more than tenfold.

Burdick and Lipschuetz (1948)[521] have shown that solutions containing the ferro and ferricyanide complexes become highly toxic to fish through photodecomposition upon exposure to sunlight. Numerous investigations have shown that toxicity of free cyanide increased at reduced oxygen concentrations (Downing 1954,[525] Wuhrmann and Woker 1955,[528] Burdick et al. 1958,[520] Cairns and Scheier 1963).[522] The toxic action is known to be accelerated markedly by increased temperature (Wuhrmann and Woker 1955,[528] Cairns and Scheier 1963),[522] but the influence of temperature during long exposure has not been demonstrated. The toxicity of the nitriles (organic cyanides) to fish varied greatly. Henderson et al. (1960)[526] found marked cumulative toxicity of acrylonitrile. Lactonitrile decomposed rapidly in water yielding free cyanide, and its high toxicity evidently was due to the HCN formed.

The toxicity of cyanide to diatoms varied little with change of temperature and was a little greater in soft water than in hard water (Patrick *unpublished data* 1971).[529] For *Nitzchia linearis*, concentrations found to cause a 50 per cent reduction in growth of the population in soft water (44 mg/l Ca-Mg as $CaCO_3$) were 0.92 mg/l (CN) at 72 F, 0.30 mg/l at 82 F, and 0.28 mg/l at 86 F. For *Navicula seminulum var. Hustedtii*, the concentrations reducing growth of the population by 50 per cent in hard water (170 mg/l Ca-Mg as $CaCO_3$) were found to be 0.36 mg/l at 72 F, 0.49 mg/l at 82 F, and-0.42 mg/l at 86 F. Cyanide appeared to be more toxic to animals than to algae.

Recommended maximum concentrations of cyanide-bearing wastes of unknown composition and properties should be determined by static and flow-through bioassays. The bioassays should be performed with dissolved oxygen, temperature, and pH held at the local water quality conditions under which cyanides are most toxic. Because the partial dissociation of some complex metallocyanide ions may be slow, static bioassays may reveal much greater toxicity than that demonstrable by the flow-through methods. On the other hand, standing test solutions of simple and some complex cyanides exposed to the atmosphere gradually lose their toxicity, because the volatile HCN escapes.

Chemical determination of the concentration of undissociated, molecular HCN alone may be the best way to evaluate the danger of free cyanide to fish in waters receiving cyanide bearing wastes. Such tests may reveal the occurrence of harmful concentrations of HCN not predictable through bioassay of the wastes. Because an acceptable concentration of HCN or fraction of a LC50 of cyanides and cyanide-bearing effluents has not yet been positively determined, a conservative estimate must be made; and because levels as low as 0.01 mg/l have proved harmful under some conditions, a factor of 0.05 should be applied to LC50 levels.

Recommendation

Once a 96-hour LC50 has been determined using the receiving water in question and the most sensitive important species in the locality as the test organism, a concentration of free cyanide (CN^-) safe to aquatic life in that water can be estimated by multiplying the 96-hour LC50 by an application factor of 0.05; but no concentration greater than 0.005 mg/l is recommended at any time or place.

Detergents

Detergents are a common component of sewage and industrial effluents derived in largest amounts from household cleaning agents. In 1965 a shift from tetrapropylene-derived alkylbenzene sulfonates (ABS) to the more biodegradable linear alkylate sulfonates (LAS) was made by the detergent industry. In current detergent formulas, LAS is the primary toxic active compound, two to four times more toxic than ABS (Pickering 1966).[534] However, toxicity of LAS disappears along with the methylene blue active substances (MBAS) response upon biodegradation (Swisher 1967).[537] Retrieval of MBAS data from the National Surveillance Stations throughout the U.S. from 1966 to the present showed that the mean of 3,608 samples was less than 0.1 mg/l. There has been a downward trend in MBAS concentrations. Only four stations reported mean concentrations greater than 0.2 mg/l.

The MBAS determination has been the routine analytical method for measurement of surfactant concentrations. Positive errors are more common than negative ones in the determination of anionic surfactants in water (Standard Methods 1971).[536] An infrared determination or a carbon absorption cleanup procedure is recommended when high MBAS concentrations are found.

Marchetti (1965)[533] critically reviewed the effects of detergents on aquatic life. Most available information on LAS toxicity relates to fish. Short term studies by a number of investigators have shown that lethal concentrations to selected fish species vary from 0.2 to 10.0 mg/l (Hokanson and Smith 1971).[532] Bardach et al. (1965)[531] reported that 10 mg/l is lethal to bullheads (*Ictalurus* sp.), and that 0.5 mg/l eroded 50 per cent of their taste buds within 24 days. Thatcher and Santner (1966)[538] found 96-hour LC50 values from 3.3 to 6.4 mg/l for five species of fish.

Pickering and Thatcher (1970)[535] found in their study of chronic toxicity that a concentration of 0.63 mg/l had no measurable effect on the life cycle of the fathead minnow (*Pimephales promelas*), while a concentration of 1.2 mg/l was lethal to the newly hatched fry. A safe level should be between 14 and 28 per cent of the 96-hour LC50. Hokanson and Smith (1971)[529] reported that a concentration of 1 mg/l was an approximate safe concentration for bluegills in Mississippi River water of good quality. Arthur (1970)[530] found that the no-effect level of LAS on *Gammarus pseudolimnaeus* was 0.2 to 0.4 mg/l. This investigator also subjected opculate and pulmonate snails to 60-week exposures of LAS and showed the toxicity levels to be 0.4 to 1.0 mg/l and greater than 4.4 mg/l, respectively.

Detergent Builders

Phosphates have been included in household detergents to increase their effectiveness, although this use has been seriously questioned recently. Nitrilotriacetate (NTA) and other builders have been tried, but most are either less effective or have been barred for reasons of potential health hazard. Available builders do not have serious direct effects on fish or aquatic organisms at concentrations likely to be encountered in receiving waters. In view of the uncertain legal status of present commercial detergents and the extensive search for adequate substitutes now in progress, recommendations for builders are not practical at this time. However, it can be stated that a satisfactory builder should be biologically degradable and nontoxic to aquatic organisms and humans, and that it should not cause aesthetic problems in the receiving water.

Recommendation

Once a 96-hour LC50 has been determined using the receiving water in question and the most sensitive important species in the locality as the test organism, a concentration of LAS safe to aquatic life in that water can be estimated by multiplying the 96-hour LC50 by an application factor of 0.05; but no concentration greater than 0.2 mg/l is recommended at any time or place.

Phenolics

Phenols and phenolic wastes are derived from petroleum, coke, and chemical industries; wood distillation; and domestic and animal wastes. Many phenolic compounds are more toxic than pure phenol: their toxicity varies with the combinations and general nature of total wastes. Acute toxicity of pure phenol varies between 0.079 mg/l in 30 minutes to minnows, and 56.0 mg/l in 96 hours to mosquito fish (*Gambusia affinis*). Mitrovic et al. (1968)[541] found a 48-hour LC50 of 7.5 mg/l to trout; they noted that exposure to 6.5 mg/l caused damage to epithelial cells in 2 hours, and extensive damage to reproductive systems in 7 days. Ellis (1937)[539] reported 1.0 mg/l safe to trout; and 0.10 mg/l was found nonlethal to bluegill (*Lepomis macrochirus*) in 48 hours (Turnbull et al. 1954).[542] These studies illustrated the wide range of phenol toxicity. There is not yet adequate documentation about chronic effects and toxicity of mixed wastes on which to base recommendations of safe levels for fish.

Phenolics affect the taste of fish at levels that do not appear to affect fish physiology adversely. Mixed wastes often have more objectionable effects than pure materials. For example, 2,4-dicholorphenol affects taste at 0.001 to 0.005 mg/l; *p*-chlorophenol at 0.01 to 0.06 mg/l; and 2-methyl, 6-chlorophenol at 0.003 mg/l. (See the discussion of Tainting Substances, p. 147.) Pure phenol did not affect taste until levels of 1 to 10 mg/l were reached (Fetterolf 1964).[540] The taste of fish in most polluted situations is adversely affected by phenolics before acute toxic effects are observed.

Recommendations

In view of the wide range of concentrations of phenolics which produce toxic effects in fish and the generally lower levels which taint fish flesh, it is recommended that taste and odor criteria be used to determine suitability of waste receiving waters to support usable fish populations. Where problems of fish kills occur or fish are subjected to occasional short-term exposure to phenolic compounds, a 96-hour LC50 should be determined using the receiving water in question and the most sensitive important fish in the locality as the test animal. Concentrations of phenolic compounds safe to fish in that water can then be estimated by multiplying the 96-hour LC50 by an application factor of 0.05; but no concentration greater than 0.1 mg/l is recommended at any time or place. Tests of other species will be necessary to protect other trophic levels.

Sulfides

Sulfides are constituents of many industrial wastes, such as those from tanneries, paper mills, chemical plants, and gas works. Hydrogen sulfide may be generated by the anaerobic decomposition of sewage and other organic matter in the water, and in sludge beds. Natural production of H_2S may also result from deposits of organic material.

When soluble sulfides are added to water, they react with hydrogen ions to form HS^- or H_2S, the proportion of each depending on the pH values. The toxicity of sulfides derives primarily from H_2S rather than the sulfide ion. The rapid combination of H_2S with other materials, including oxygen, has frequently caused investigators to overlook the importance of H_2S as it affects aquatic life, especially when it originates from sludge beds. Because water samples usually are not taken at the mud/water interface, the importance of H_2S in this habitat for fish eggs, fish fry, and

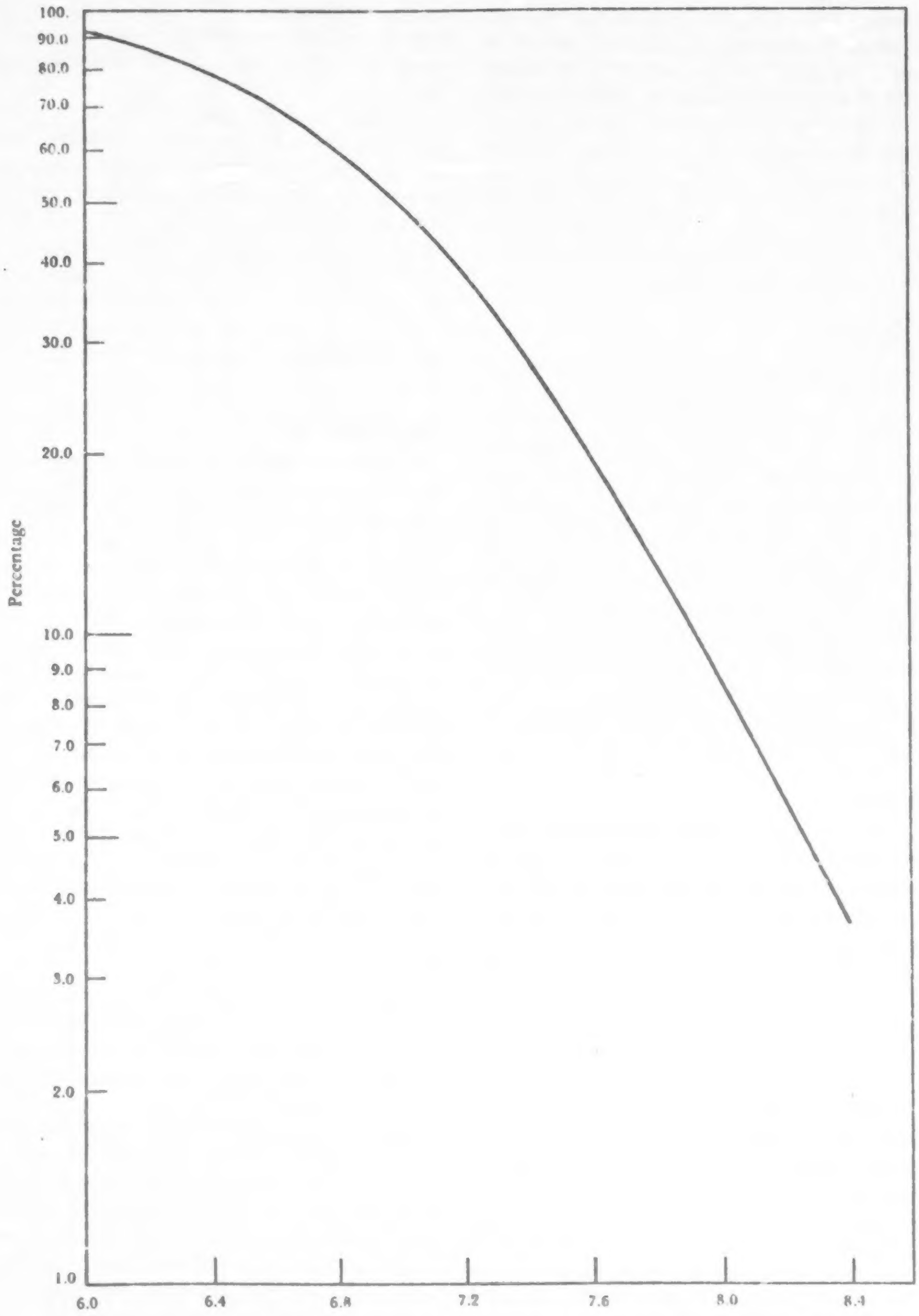


FIGURE III-11—Percentage of Hydrogen Sulfide in the Form of Undissociated H_2S at Various pH Levels (Temperature = 20 C; ionic strength μ = 0.01)

fish food organisms is often overlooked (Colby and Smith 1967).[545]

Hydrogen sulfide is a poisonous gas, soluble in water to the extent of about 4,000 mg/l at 20 C and one atmosphere of pressure (Figure III–11). Upon solution, it dissociates according to the reaction $H_2S \rightarrow HS^- + H^+$ and $HS^- \rightarrow S^{--} + H^+$. At pH 9, about 99 per cent of the sulfide is in the form of HS^-; at pH 7 it is about equally divided between HS^- and H_2S; and at pH 5 about 99 per cent is present as H_2S.

Consequently, the toxicity of sulfides increases at lower pH because a greater proportion is in the form of undissociated H_2S. Only at pH 10 and above is the sulfide ion present in appreciable amounts. In polluted situations, where the pH may be neutral or below 7.0, or where oxygen levels are low but not lethal, problems arising from sulfides or from hydrogen sulfide generated in sludge deposits will be increased.

Much available data on the toxicity of hydrogen sulfide to fish and aquatic life have been based on extremely short exposure periods and have failed to give adequate information on water quality, oxygen, and pH. Consequently, early data have suggested that concentrations between 0.3 and 4.0 mg/l permit fish to survive (Schaut 1939,[546] VanHorn 1958,[550] Bonn and Follis 1967,[544] Theede et al. 1969).[549] Recent data both in field situations and under controlled laboratory conditions demonstrated hydrogen sulfide toxicity at lower concentrations. Colby and Smith (1967)[545] found that concentrations as high as 0.7 mg/l were found within 20 mm of the bottom on sludge beds, and that levels of 0.1 to 0.02 mg/l were common within the first 20 mm of water above this layer. Walleye (*Stizostedion vitreum v.*) eggs held in trays in this zone did not hatch. Adelman and Smith (1970)[543] reported that hatching of northern pike (*Esox lucius*) eggs was substantially reduced at 0.025 mg/l of H_2S, and at 0.047 mg/l mortality was almost complete. Northern pike fry had 96-hour LC50 values that varied from 0.017 to 0.032 mg/l at normal oxygen levels (6.0 mg/l). The highest concentration of hydrogen sulfide at which no short-term effects on eggs or fry were observed was 0.814 mg/l. Smith and Oseid (*in press* 1971),[548] working on eggs, fry, and juveniles of walleyes and white suckers (*Catostomus commersonni*), and Smith (1971),[547] working on walleyes and fathead minnows (*Pimephales promelas*), found that safe levels varied from 0.0029 to 0.012 mg/l with eggs being the least sensitive and juveniles being the most sensitive in short-term tests (Table III–20). In 96-hour bioassays fathead minnows and goldfish (*Carassius auratus*) varied greatly in tolerance to hydrogen sulfide with changes in temperature. They were more tolerant at low temperatures (6 to 10 C).

TABLE III-20—96-Hour LC50 and Safe Levels Based on No Adverse Effect on Critical Life History Stages

Species		96-Hr. LC50 (mg/l)	Safe levels* (mg/l)
Northern Pike	eggs	0.037	0.014
	fry	0.0[illegible]	0.004
Walleye	eggs	0.071	0.012
	fry	0.007	0.007
	juvenile	0.017	0.0037
White Sucker	eggs		0.015
	fry	0.0018	0.002
	juvenile	0.0185	0.002
Fathead minnows	juvenile	0.032 (at 20 C)	0.003
	adult	0.032	0.003
Bluegill	juvenile	0.032	0.002
	adult	0.032	0.002
Gammarus pseudolimnaeus		0.042 (10 day)	0.0033
Hexagenia limbata		0.350	

* Safe levels are construed to mean no demonstrable deleterious effect on survival or growth after long-term chronic exposure.

On the basis of chronic tests evaluating growth and survival, the safe level for bluegill (*Lepomis macrochirus*) juveniles and adults was 0.002 mg/l. White sucker eggs all hatched at 0.015 mg/l, but juveniles showed a negligible growth reduction at 0.002 mg/l. Safe levels for fathead minnows were between 0.002 and 0.003 mg/l. Studies on various arthropods (*Gammarus pseudolimnaeus* and *Hexagenia limbata*), useful as fish food, indicated that safe levels were between 0.002 and 0.003 mg/l (Smith 1971).[547] Some species typical of normally stressed habitats were much more resistant (*Asellus* sp.).

Recommendation

On the basis of available data, a level of undissociated hydrogen sulfide assumed to be safe for all aquatic organisms including fish is 0.002 mg/l. At a pH of 6.0 and a temperature of 13.0 C, approximately 99 per cent of the total sulfide is present as undissociated hydrogen sulfide. Therefore, to protect aquatic organisms within the acceptable limits of pH and temperature, it is recommended that the concentration of total sulfides not exceed 0.002 mg/l at any time or place.

WILDLIFE

In this report, wildlife is defined as all species of vertebrates other than fish and man. To assure the short-term and long-term survival of wildlife, the water of the aquatic ecosystem must be of the quality and quantity to furnish the necessary life support throughout the life-cycle of the species involved. In addition to the quantity, the quality of food substances produced by the aquatic environment must be adequate to support the long-term survival of the wildlife species.

Many species of wildlife require the existence of specific, complex, and relatively undisturbed ecosystems for their continued existence. Aquatic ecosystems, such as bogs, muskegs, seepages, swamps, and marshes, can exhibit marked fragility under the influence of changing water levels, various pollutants, fire, or human activity. Changes in the abundance of animal populations living in such aquatic communities can result in reactions and altered abundance of plant life, which in turn will have repercussions of other species of animal life. In general, these transitional ecosystems between land and water are characterized by very high productivity and importance for wildlife, and they should thus be maintained in that state to the greatest possible extent.

In many instances, criteria to protect fish and invertebrates or to provide water suitable for consumption by man or domestic animals will also provide the minimal requisites for some species of wildlife. This would be true for species that use water only for direct consumption or that feed on aquatic organisms to only a minor extent. For many species of wildlife, however, the setting of water quality criteria is complicated by their ecological position at the apex of complex food webs, and also by the extreme mobility of some wildlife, especially birds.

Those substances which are concentrated via food chains, such as many chlorinated hydrocarbons, present special problems for those species that occupy the apex of long food chains. In those instances, environmental levels which are safe for fish, do not necessarily convey safety to predators or even to scavengers that consume fish.

PROTECTION OF FOOD AND SHELTER FOR WILDLIFE

A number of factors can be identified that can affect specific components of the ecosystem and cause reduced food and shelter for wildlife. These factors also affect fish and other aquatic life and therefore are discussed in greater detail in appropriate related subtopics.

pH

In bioassays with aquatic plants, Sincock (1968)[595] found that when the pH of the water in test vessels dropped to 4.5, reedhead-grass (*Potamogeton perfoliatus*), a valuable waterfowl food plant, died within a few days. Similarly, in Back Bay, Virginia, between August and November, 1963, the aquatic plant production declined from 164 to 13 pounds per acre. This atypical decline was immediately preceded by a decline in pH to 6.5 compared to previous midsummer readings of 7.7 to 9.2. (U.S. Bureau of Sport Fisheries and Wildlife).[601]

Recommendation

Aquatic plants of greatest value as food for waterfowl thrive best in waters with a summer pH range of 7.0 to 9.2.

ALKALINITY

Generally, waters with reasonably high bicarbonate alkalinity are more productive of valuable waterfowl food plants than are waters with low bicarbonate alkalinity. Few waters with less than 25 mg/1 bicarbonate alkalinity can be classed among the better waterfowl habitats. Many waterfowl habitats productive of valuable foods, such as sago pondweed (*Potamogeton pectinatus*), widgeongrass (*Rappia maritima* and *R. occidentalis*), banana waterlily (*Castalia flava*), wild celery, (*Vallisneria americana*), and others have a bicarbonate alkalinity range of 35 to 200 mg/1.

Definitive submerged aquatic plant communities develop in waters with different concentrations of bicarbonate alkalinity. It is logical to assume that excessive and prolonged fluctuation in alkalinity would not be conducive to stabilization of any one plant community type. Sufficient experimental evidence is not available to define the effects of various degrees and rates of change in alkalinity on aquatic plant communities. Fluctuations of 50 mg/l probably would contribute to unstable plant communities. Fluctuations of this magnitude may be due to canals connecting watersheds, diversion of irrigation water, or flood diversion canals (Federal Water Pollution Control Administration 1968, hereafter referred to as FWPCA 1968).[562]

Recommendation

Waterfowl habitats should have a bicarbonate alkalinity between 30 and 130 mg/l to be productive. Fluctuations should be less than 50 mg/l from natural conditions.

SALINITY

Salinity can also affect plant communities. All saline water communities, from slightly brackish to marine, produce valuable waterfowl foods, and the most important consideration is the degree of fluctuation of salinity. The germination of seeds and the growth of seedlings are critical stages in the plant-salinity relationship; plants become more tolerant to salinity with age.

Salinities from 0.35 to 0.9 per cent NaCl in drinking water have been shown to be toxic to many members of the order Galliformes (chickens, pheasant, quail) (Krista et al. 1961,[565] Scrivner 1946,[572] Field and Evans 1946[561]).

Young ducklings were killed or retarded in growth as a result of salt poisoning by solutions equal to those found on the Suisun Marsh, California, during the summer months. Salinity maxima varied from 0.55 to 1.74 per cent, and the means varied from 0.07 to 1.26 per cent during July from 1956 to 1960 (Griffith 1962–63).[563]

Recommendation

Salinity should be kept as close to natural conditions as possible. Rapid fluctuations should be minimized.

LIGHT PENETRATION

Criteria for light penetration established in the discussions of Color (p. 130) and Settleable Solids (p. 129) should also be adequate to provide for the production of aquatic plants for freshwater wildlife.

SETTLEABLE SUBSTANCES

Accumulation of silt deposits are destructive to aquatic plants due especially to the creation of a soft, semi-liquid substratum inadequate for the anchoring of roots. Back Bay, Virginia, and Currituck Sound, North Carolina, serve as examples of the destructive nature of silt deposition. Approximately 40 square miles of bottom are covered with soft, semi-liquid silts up to 5 inches deep; these areas, constituting one-fifth of the total area, produce only 1 per cent of the total aquatic plant production (FWPCA 1968).[562]

Recommendation

Setteable substances can destroy the usefulness of aquatic bottoms to waterfowl, and for that reason, settleable substances should be minimized in areas expected to support waterfowl.

PRODUCTION OF WILDLIFE FOODS OTHER THAN PLANTS

The production of protozoans, crustaceans, aquatic insects, other invertebrates, and fish is dependent on water quality. The water quality requirements for the production of fish are dealt with elsewhere in this Section, and a normal level of productivity of invertebrates is also required for the normal production of fish that feed upon them.

While it is well known that many species of invertebrates are easily affected by low concentrations of pollutants, such as insecticides, in water (Gaufin et al. 1965,[563] Burdick et al. 1968,[555] Kennedy et al. 1970[564]), most of the field studies do not supply reliable exposure data, and most laboratory studies are of too short a duration or are performed under static conditions, allowing no reliable extrapolations to natural conditions. The general impression to be gained from these studies is that insects and crustaceans tend to be as sensitive as or more sensitive than fish to various insecticides, and that many molluscs and oligochetes tend to be less sensitive.

TEMPERATURE

The increasing discharge of warmed industrial and domestic effluents into northern streams and lakes has changed the duration and extent of normal ice cover in these northern regions. This has prompted changes in the normal overwintering pattern of some species of waterfowl. Thus, Hunt (1957)[576] details the increasing use since 1930 of the Detroit River as a wintering area for black duck (*Anas rubripes*), canvasback (*Aythya valisneria*), lesser scaup (*Aythya affines*), and redhead (*Athya americana*). In this process, waterfowl may become crowded into areas near industrial complexes with a shrinking supply of winter food. The proximity of sources of pollutants, food shortages, and low air temperatures often interact to produce unusually high waterfowl mortalities.

Recommendation

Changes in natural freezing patterns and dates should be avoided as far as possible in order to minimize abnormal concentrations of wintering waterfowl.

SPECIFIC POTENTIALLY HARMFUL SUBSTANCES

Direct Acting Substances

Oils Waterbirds and aquatic mammals, such as muskrat and otter, require water that is free from surface oil. Catastrophic losses of waterbirds have resulted from the contamination of plumages by oils. Diving birds appear to be more susceptible to oiling than other species (Hawkes 1961).[571] Heavy contamination of the plumage results in loss of buoyancy and drowning. Lower levels of contamination cause excessive heat loss resulting in an energy deficit which expresses itself in an accelerated starvation (Hartung 1967a).[567] Less than 5 mg of oil per bird can produce significant increases in heat loss. The ingestion of oils may contribute to mortalities, and this is especially true for some manufactured oils (Hartung and Hunt 1966).[569] When small quantities of oil are coated onto eggs by incubating mallards (*Anas platyrhynchos*), the likelihood of those eggs to hatch is greatly reduced (Hartung 1965).[566] Rittinghaus (1956)[581] reported an incident in which numerous Cabot's Terns (*Thalasseus sandvicensis*) and other shorebirds became contaminated with oil that had been washed on shore. Eggs which were subsequently oiled by the plumage of oiled female terns did not hatch even after 50 days of incubation. The absence of visible surface oils should protect wildlife from direct effect.

Oils can be sedimented by coating particulates on the surface and then sinking to the bottom. Sedimented oils have been associated with changes in benthic communities (Hunt 1957)[576] and have been shown to act as concentrators for chlorinated hydrocarbon pesticides (Hartung and Klingler 1970[570]).

Recommendation

To protect waterfowl, there should be no visible floating oil (see p. 146 of this Section and pp. 263–264 of Section IV).

Lead Waterfowl often mistake spent lead shot for seed or grit and ingest it. See Section IV, pp. 227–228, for a discussion of this problem.

Recommendation

The recommendation of the Marine Aquatic Life and Wildlife Panel, Section IV, (p. 228) to protect waterfowl also applies to the freshwater environment.

Botulism Poisoning Botulism is a food poisoning caused by the ingestion of the toxin of *Clostridium botulinum* of any six immunologically distinct types, designated A through F. The disease, as it occurs in epizootic proportions in wild birds, is most commonly of the C type, although outbreaks of type E botulism have been observed on the Great Lakes (Kaufman and Fay 1964[582], Fay 1966[560]).

Cl. botulinum, a widely distributed anaerobic bacterium, is capable of existing for many years in its dormant spore form, even under chemically and physically adverse environmental conditions. Its toxins are produced in the course of its metabolic activity as the vegetative form grows and reproduces in suitable media. Outbreaks occur when aquatic birds consume this preformed toxin.

The highest morbidity and mortality rates from botulism in aquatic birds have been recorded in shallow, alkaline lakes or marshes in the western United States, and outbreaks have most commonly occurred from July through September and, in some years, October. The optimum temperatures for growth of the bacterium or the toxin production, or both, have been reported as low as 25 C (Hunter et al. 1970)[577] and as high as 37 C (Quortrup and Sudheimer 1942[588]). The discrepancies are probably the result of differences in the experimental conditions under which the measurements were made and the strains of *Cl. botulinum* type C used.

The popular belief that avian botulism epizootics are associated with low water levels and consequent stagnation is not necessarily supported by facts. In three of the years of heaviest bird losses in the history of the Bear River Migratory Bird Refuge (1965, 1967, and 1971), the water supply was considerably more abundant than normal (Hunter, California Department of Fish and Game, *personal communication*; unpublished Bureau of Sport Fisheries and Wildlife reports[600]). The high water levels caused flooding of mud flats not normally under water in the summer months. Similar inundations of soil that had been dry for several years have been associated previously with outbreaks on the Bear River Refuge and in other epizootic areas. A partial explanation for these associations may be that flooding of dry ground is commonly followed by a proliferation of many species of aquatic invertebrates (McKnight 1970[587]), the carcasses of which may be utilized by *Cl. botulinum*.

Bell et al. (1955)[552] provided experimental support for an idea expressed earlier by Kalmbach (1934).[551] According to their "microenvironment concept," the bodies of invertebrate animals provide the nutrients and the anaerobic environment required by *C. botulinum* type C for growth and toxin production. These bodies would presumably also offer some protection to the bacterium and its toxin from a chemically unfavorable ambient medium. Jensen and Allen (1960)[578] presented evidence of a possible relationship between die-offs of certain invertebrate species and subsequent botulism outbreaks.

The relationship between alkalinity or salinity of the marsh and the occurrence of botulism outbreaks is not clear. Invertebrate carcasses suspended in distilled water support high levels of toxin (Bell et al. 1955).[552] Laboratory media are commonly composed of ingredients such as peptones, yeast extract, and glucose, without added salts. The medium used routinely at the Bear River Research Station for the culture of *Cl. botulinum* type C has a pH of 6.8 to 7.0 after heat sterilization. McKee et al. (1958)[556] showed that when pH was automatically maintained at a particular level in laboratory cultures of *Cl. botulinum* type C throughout the growth period, the largest amount of toxin was produced at pH 5.7, the lowest level tested. Decomposing carcasses of birds dead of botulism commonly contain very high concentrations of type C toxin, and in these cases production is ordinarily independent of the chemical composition of the marsh.

Kalmbach (1934)[551] tabulated the salt concentrations of water samples collected from 10 known botulism epizootic areas. The values ranged from 261 to 102,658 ppm (omitting the highest, which was taken from a lake where the bird losses were possibly from a cause other than botulism).

Christiansen and Low (1970)[558] recorded conductance measurements on water in the management units of the Bear River Migratory Bird Refuge and the Farmington Bay Waterfowl Management Area, both sites of botulism outbreaks varying in severity from year to year. The average conductance of water flowing into the five units of the Bear River Refuge in five summers (1959–1963) ranged from 3.7 to 4.9 millimhos per centimeter at 25 C. The readings on outflowing water from the five units ranged from 4.4 to 8.3 mmhos. Comparable figures for the three Farmington Bay units were 1.8 to 3.2 (inflow) and 3.2 to 4.8 mmhos (outflow). Thus the salinity range of the inflowing water at Bear River was comparable to that of the outflowing water at Farmington.

These data suggest that salt concentration of the water in an epizootic area is not one of the critical factors influencing the occurrence of outbreaks. If high salinity does favor their occurrence, it is probably not because of its effect on *Cl. botulinum* itself. Other possible explanations for the higher incidence of botulism in shallow, alkaline marshes are:

- Saline waters may support higher invertebrate population levels than do relatively fresh waters. (Comparisons, as they relate to avian botulism, have not been made.)
- High salinity may inhibit some of the microorganisms that compete with *Cl. botulinum* for nutrients or those that cause deterioration of the toxin.
- Salinity may have no significant effect on the invertebrates or the bacteria, but it increases the susceptibility of the birds. Cooch (1964)[557] has shown that type C botulinum toxin decreases the activity of the salt gland in ducks, reducing its capacity to eliminate salt. Birds so affected succumb to smaller doses of toxin than do those provided with fresh water.
- Outbreaks of botulism poisoning tend to be associated with or affected by insect die-offs, water temperatures above 70 F, fluctuations in water levels and elevated concentrations of dissolved solids.

Recommendation

Outbreaks of botulism poisoning tend to be associated with, or affected by insect die-offs, water temperature above 70 F, fluctuating water levels, and elevated concentrations of dissolved solids. Management of these factors may reduce outbreaks of botulism poisoning.

Substances Acting After Magnification in Food Chains

Chlorinated Hydrocarbon Pesticides

DDT and Derivatives DDT and its abundant derivatives DDE and TDE have high lipid solubility and low water solubility, and thus tend to concentrate in the lipid, i.e., living fraction of the aquatic environment (Hartung 1967b).[568] DDE is the most stable of the DDT compounds and has been especially implicated in producing thinning of egg shells, increased breakage of eggs, reproductive failure in species occupying the apex of aquatic food chains in areas with long histories of DDT usage.

Reproductive failures and local extirpation associated with egg shell thinning have been reported for several North American bird species. The phenomenon was first described and is most wide-spread for the peregrine falcon (*Falco peregrinus*) (Hickey and Anderson 1968).[574] Since then similar phenomena have been described in Brown Pelicans (*Pelecanus occidentalis*) (Anderson and Hickey 1970)[531] and species of several other families of predatory birds. Further increases of DDE in large receiving basins, such as the Great Lakes, would be expected to increase the extent of reproductive failure among predatory aquatic bird populations. Concentrations as low as 2.8 ppm *p,p'*DDE on a wet-weight basis produced experimental thinning of egg shells in the American Kestrel (*Falco sparvarius*) (Wiemeyer and Porter 1970).[599] Heath et al. (1969)[572] induced significant levels of eggshell thinning in mallards after feeding them similarly low levels of DDE. Concentrations of DDT compounds in the water of Lake Michigan have been estimated to be 1 to 3 parts per trillion (Reinert 1970)[589] (Table III-21). Concentrations that would permit the assured survival of sensitive predatory bird species are evidently much lower than that. Because such low concentrations cannot be reliably measured by present technologies and because the concentrating factor for the food chains appears to be variable or is not known, or both, a biological monitoring system should be chosen. If it is desired to protect a number of fish-eating and raptorial birds, it is essential to reduce the levels of DDE contamination, especially in large receiving basins (see Section IV).

The available data indicate that there should not be concentrations greater than 1 mg/kg of total DDT in any aquatic plants or animals in order to protect most species of aquatic wildlife. Present unpublished data indicate effects for even lower levels of DDE to some species of predatory birds (Stickel *unpublished data*).[601]

Present environmental levels vastly exceed the recommended levels in many locations, and continued direct or

TABLE III-21—Relationship of DDT and Metabolites to Eggshell Thinning

Species	Dosage* wet-weight basis	Pesticide level in eggs	Thinning Percent	Reference
Mallard	1000 mg/kg single dose	N.D.†	25	Tucker & Haegele, 1970
Prairie falcon (Falco mexicanus)	N.D.†	5-10 ppm DDE	ca. 5	Enderson & Berger, 1970
		13-23 ppm DDE	ca. 13	
		25-30 ppm DDE	ca. 18	
		30 ppm DDE	ca. 25	
Japanese quail (Coturnix)	100 ppm o,pDDT	23.0 ppm o,pDDT 8.57 ppm DDE	4	Bitman et al., 1969
	100 ppm p,pDDT	40.1 ppm p,pDDE	8	
Herring gull (Larus argentatus)	ca. 3.3 ppm total DDT	227 ppm total DDT	N.D.†	Keith, 1966
American kestrel (Falco sparverius)	2.8 ppm p,pDDE	32.4 ppm DDE	10	Wiemeyer & Porter, 1970
Mallard	**2.8 ppm DDE	N.D.†	11	Heath et al., 1969
	**11.2 ppm DDE	N.D.†	14	

* All tests except the first one are chronic, spanning at least several months.
** Converted from dry-basis.
† Not determined.

indirect inputs of DDT would make these recommendations unattainable.

Recommendation

In order to protect most species of aquatic wildlife, the total DDT concentration on a wet-weight basis should be less than 1 mg/kg in any aquatic plants or animals. (Also see Recommendations for Pesticides, p. 185-186.)

Polychlorinated Biphenyls (PCB) Polychlorinated biphenyls are chlorinated hydrocarbons which are highly resistant to chemical or biological degradation. They have been widespread environmental contaminants (Jensen et al. 1969,[580] Risebrough et al. 1968[586]). Their biological effects at present environmental concentrations are not known. PCB's can elevate microsomal enzyme activity (Risebrough et al. 1968,[586] Street et al. 1968[591]), but the environmental significance of that finding is not clear. The toxicity of PCB is influenced by the presence of small amounts of contaminated chlorinated dibenzofurans (Vos and Koeman 1970,[595] Vos et al. 1970[597]) which are highly toxic to developing embryos.

Recommendation

Because of the persistence of PCB and their susceptibility to biological magnification, it is recommended that the body burdens of PCB in birds and mammals not be permitted to increase and that monitoring programs be instituted (see Section IV).

Mercury

Westoo (1966)[593] reported that almost all of the mercury found in fish is methyl mercury. Jensen and Jernelöv (1969)[579] showed that natural sediments can methylate ionic mercury. Mercury levels in fish in Lake St. Clair ranged between 0.4 and 3 ppm, averaging near 1.5 ppm (Greig and Seagram 1970).[564] Residues in fish-eating birds from Lake St. Clair ranged up to 7.5 ppm in a tern, and up to 23 ppm in a great blue heron (Dustman et al. 1970).[555] These residues are comparable to those found in Swedish birds that died after experimental dosing with methylmercury, and in birds that died with signs of mercury poisoning under field conditions in Scandinavian countres (Henriksson et al. 1966,[573] Borg et al. 1969,[554] Holt 1969[576]). To date, no bird mortalities due to mercury contamination have been demonstrated in the Lake St. Clair area, but body burdens of fish-eating birds are obviously close to demonstrated toxic levels. It is therefore concluded that the mercury levels in fish flesh should be kept below 0.5 ppm to assure the long-term survival of fish-eating birds. Since this level incorporates little or no safety margin for fish-eating wildlife, it is suggested that the safety of a 0.5 ppm level be reevaluated as soon as possible.

Recommendation

Fish-eating birds should be protected if mercury levels in fish do not exceed 0.5 μg/g.

Since the recommendation of 0.5 μg/g mercury in fish provides little or no safety margin for fish-eating wildlife, it is recommended that the safety of the 0.5 μg/g level be reevaluated as soon as possible.

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INTRODUCTION

The Panel on Marine Aquatic Life and Wildlife took as its prime responsibility the development of recommendations that would reasonably assure protection of the marine ecosystem. The recommendations have been discussed at various meetings of the members of the Panel and represent a consensus on the best statement that can be made in the light of present knowledge. The recommendations are not inflexible and may be modified as our understanding of the marine ecosystem improves.

Many parts of the marine ecosystem do not meet the quality requirements recommended here. As a result of man's activities, the marine ecosystem has been greatly modified; many species are excluded from areas where they were once abundant, and many areas have been closed for the harvesting of marine products as human food because of pollution. The decision as to what part, and how much, of the marine ecosystem should be protected for normal aquatic life and wildlife has political, social, and economic aspects, and such decisions cannot be based upon scientific evidence alone. Although some marine pollution problems are local in character, many are global and only the broadest possible approach can solve these problems. Food from the sea is already an important source of animal protein for human nutrition, and this continuing supply must not be diminished by pollution.

At the same time, the Panel recognizes that additions of pollutants to the oceans as by-products of our present mode of living will continue. But if pollution is kept within the boundaries and constraints which are defined in the recommendations, the Panel believes that the marine ecosystem can be protected.

In many ways the marine ecosystem is similar to the freshwater, but there are significant differences which should be briefly described. For more details which summarize the extensive literature on this subject, the reader is referred to *The Oceans* by Sverdrup, et al. (1942)[8],* *The Sea*, particularly volume 2 edited by M. N. Hill (1964),[3] and *Estuaries*, edited by G. H. Lauff (1967).[4]

The marine environment is a significant source of animal protein with an annual production of about 60 million tons fresh weight of fisheries products (Food and Agriculture Organization 1967).[2] Various estimates of the potential expansion of this harvest have been made and are summarized by Ryther (1969)[6] who concludes that the potential harvest might double this figure. Some of the existing stocks are already fished to capacity or overfished, but aquaculture (pp. 222–224) may increase world marine production.

The importance of this supply of animal protein to the world population has been emphasized by Borgstrom (1961).[1] He estimates that more than two billion people of the world's population receive 50 per cent or more of their animal protein from marine products. In the United States fish contributes only about 5 per cent of our animal protein consumption, but even so it has been estimated by Pruter (*unpublished* 1972)[5] that over ten billion pounds of commercial fish and shellfish were harvested from the estuaries and continental shelf of the United States in 1970. Furthermore, in the United States a great deal of fishmeal is used to fortify animal feeds, particularly for chickens. It is obvious that this valuable food resource of the marine environment must be sustained.

The estuaries are regions where the impact of man's activity is greatest, and they are also areas of great value for marine fish production. They serve not only as nursery areas and breeding grounds for many species of fish, but also as the regular home for the entire life cycle of some valuable species, such as oysters and crabs. Sykes (1968)[7] has estimated that 90 per cent or more of the commercial catch of finfish in some geographical regions of the United States consists of estuarine-dependent species. The estuaries are the most variable regions of the marine ecosystem (see pp. 219–221) and organisms which inhabit them are exposed to extreme variations. Since these organisms survive, they are obviously adapted to the stress imposed by these variations. During the tidal cycle, a sessile organism will be exposed to variations in temperature and salinity as the tide ebbs and flows. On a seasonal basis, because of variations in river flow, organisms at a fixed location may be exposed to fresh water during flood periods or to nearly undiluted sea water during droughts. The oscillatory nature of the tidal currents can also lead to an accumulation of pollutants within an estuary, as is discussed in the section on waste capacity of receiving waters (pp. 228–232).

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* Citations are listed at the end of the Section. They can be located alphabetically within subtopics or by their superior numbers which run consecutively across subtopics for the entire Section.

Migratory fishes must also pass through estuaries in order to reach their breeding grounds. Anadromous fishes, such as the alewife, salmon, shad, and striped bass, move upstream to breed in the highly diluted seawater or in fresh water. In contrast the catadromous species such as the eel spend their adult stages in fresh water and migrate downstream in order to breed in the open sea. Conditions within the estuaries should be maintained so that these seasonal breeding migrations are not interfered with.

The conditions in the coastal waters are less variable than those in the estuaries, but in temperate regions, the seasonal range of conditions can be considerable. The coastal waters, particularly in areas of upwelling, are the most highly productive parts of the marine environment and have been estimated by Ryther (1969)[8] to produce half of the potential marine fish production, even though they constitute only 0.1 per cent of the total area of the oceans. The coastal zones, including near shore areas of high production such as fishing banks, constitute 9.9 per cent of the area of the ocean and contribute nearly half of the world fish production. In tropical waters, the seasonal variation in conditions is less extreme than in temperate waters. However, as will be discussed in the section on temperature (p. 238) many tropical species are living near their upper extreme temperature during the summer, and this fact presents considerable problems in the disposal of waste heat in tropical areas.

The open ocean constitutes 90 per cent of the area of the world ocean and is the least variable of the marine environments. The deep sea produces only a minor fraction of the world's fish production, and this consists mainly of the large pelagic carnivores such as the tuna (Ryther 1969).[8] During the 19th century, the whale harvest was substantially greater than it is at present, but the whales captured were not as effectively utilized as they are in modern whaling methods. Many species of whales were grossly over-fished, and there is considerable question today whether some of these species can recover their original population sizes even in those cases where a complete moratorium on their capture is in effect.

The waters of the deep sea below the permanent thermocline (the depths below which seasonal temperature changes do not occur) constitute the largest and most constant environment on earth. During the history of modern oceanography, which covers the last century, no significant changes in either salinity or temperature of the deep sea have been observed, the organisms living in this abyssal environment having evolved under conditions which were presumably constant for millenia. To protect the coastal environment many proposals have been made to dump materials, such as solid waste, sewage sludge, and contaminated drege spoils in the deep sea. Since the organisms inhabiting the depths of the ocean have been exposed to a constant environment, they are not accustomed to unusual stresses which might be created by such dumping operations (see pp. 278–283). Consequently, dumping of organic wastes in the deep sea is not recommended (pp. 277, 282–283).

Development of Recommendations

In most cases, recommendations are not applicable to every local situation. The marine environment varies widely, and only an understanding of local conditions will make it possible to determine what can or cannot be added in each situation. Many materials are accumulated by marine organisms, and the concentration is often increased at higher levels of the food web. With substances that are toxic and persistent, it is the concentration in the highest predators, fish or birds, that is critical. One example is DDT and its derivatives which have accumulated in birds to levels that interfere with their breeding. Materials that decompose or are otherwise removed from the marine environment present lesser hazards.

The application of any recommendation to a local situation is unique because it requires (a) an understanding of the circulation of the water and the resultant mixing and dilution of the pollutant, (b) a knowledge of the local biological species in the environment and the identification of those that are most sensitive to the pollutant being considered, and (c) an evaluation of the transport of the material through the food web because of the possibility that the pollutant may reach concentrations hazardous either to the normal aquatic species present, or to man through his use of aquatic species as food.

The normal cycle of variation in the environment of many substances or conditions that occur naturally, such as oxygen, temperature, and nutrients, must be determined before decisions can be made as to possible permissible changes. In many estuaries and coastal waters "normal" conditions have been modified by man's activities and may already have changed to the extent that some species that might have been found at earlier times have been eliminated. In some circumstances, a recommendation may not be applicable because it may be necessary to specify no additional change beyond that which has already occurred. There is no generally applicable formula for recommendations to protect marine aquatic life and wildlife; a study of local environmental conditions is essential prior to application of the recommendations.

The Panel recognizes that what can or should be done in a given situation cannot wait for the completion of time-consuming studies. The degree of protection desired for a given location involves social and political decisions. The ecological nature and quality of each water mass proposed for modification must be assessed prior to any decision to modify. This requires appropriate information on the physical and chemical characteristics, on the distribution and abundance of species, and on the normal variations in these attributes over the annual cycle. In addition, there must be sufficient knowledge to permit useful prediction of the significant effects of the proposed pollutant on the stages in the life cycle of important species, on populations, and on the biological communities present. The possible impact of that pollutant upon the ecosystem can then be assessed. These subjects are covered in greater detail in other parts of this Section, but they are mentioned here to emphasize their primary importance in determining how the recommendations should be used in local situations.

USES OF THE MARINE SYSTEM TO BE PROTECTED

Coastal marine waters serve a wide variety of exceptionally important human uses. Many of these uses produce high local benefits such as the yield of shellfish and recreational activities. Others involve regional benefits or the global unity of the marine system, since local events influence, and are influenced by, water quality at distant points. Many of the human uses of marine waters are directly dependent upon the nature and quality of the biological, chemical, and physical systems present. Efforts to protect and enhance these uses will be limited principally by our ability to understand and protect the environmental conditions which are essential for the biota.

Water quality criteria for marine aquatic life and wildlife define the environmental requirements for specified uses. Five of these are discussed in this Section, namely, maintenance of the ecosystem; fisheries; aquaculture; wildlife protection; and waste disposal. These are not sharply separable, but the water quality requirements for each use are briefly summarized. The effects of transportation, harbor development, dredging and dumping of spoils have also been considered in developing the recommendations.

NATURE OF THE ECOSYSTEM

Many of the principal human uses of marine waters depend upon successful maintenance and enhancement of the existing ecosystems or, in a few circumstances, upon creating and continuing new and artificial ecosystems for specific purposes. The ecosystem includes all of the biological and non-biological (geological, physical, and chemical) components of the environment and their highly complex interactions. Studies of ecosystems must include all that is within the body of water as well as the imports to and exports from it. Research in such situations has shown that the biotic elements include producers of organic material, several levels of consumers, and decomposers. In the least complex situation, these act at rates controlled by the abiotic factors to transfer energy and recycle materials. In those aquatic environments which continuously or intermittently exchange large quantities of energy or materials with other parts of the total global system, understanding and management become more difficult. In the marine environment imports and exports continually occur from coastal runoff, tidal action, oceanic currents, meterological actions, and exchanges with adjacent water bodies or with the benthos and atmosphere. These exchanges are only partially understood, but it is clear that each marine site is connected intimately to the rest of the oceans and to total global mechanisms.

The estuaries are in many ways the most complicated and variable of aquatic ecosystems. Materials carried from the land by rivers vary in quantity and quality, sometimes with strong seasonal patterns of high biological significance. Tidal oscillations cause vigorous reversals of flow. Inherent hydrographic patterns can lead to accumulation of materials and to upstream transport from the point of addition. Dense urban populations on the shores of estuaries produce large amounts of waste, and engineering projects have changed the boundaries and flows of water courses. The biologically rich estuaries are the most variable and the most endangered part of the marine environment.

In each environment the existing characteristics of the system have been produced by dynamic interaction among the components, forces, and processes present. Some of these are small or transitory, but others are massive and enduring. If any one of these forces or processes is changed, a new balance is produced in the system. Relative stability, therefore, results from the balancing of forces, not the absence. The biota are the product of evolution, and each ecosystem contains those species and communities which have adapted to the specific environment over a long period of time and which are successful in that environment. Drastic and rapid modification of the environment, as by pollution, may eliminate some species and encourage others in ways which can reduce the value of the ecosystem for man's use or enjoyment.

Effects of Water Quality Change on Ecosystems

The introduction of a chemical compound or a change in the physical environment may affect a natural marine ecosystem in many ways. In coastal waters undisturbed for long periods of time, the ecosystem has adjusted to the existing conditions. The system is productive, species are diverse, the biomass is high, and the flow of energy is comparatively efficient. The addition of pollutants to such a system might:

- reduce the input of solar energy into the ecosystem;
- increase the input of organic matter and nutrients which might stimulate the growth of undesirable species;
- reduce the availability of nutrients by increased sorption and sedimentation;
- create intolerable physical extremes for some organisms, as by the addition of heat;
- kill or reduce the success of individual organisms, as by lethal toxicity or crippling with oil;
- eliminate species by adding a toxic material or making an essential element unavailable;
- interfere with the flow of energy from species to species, as by a chemical that interferes with feeding behavior;
- reduce species diversity in the system;
- interfere with regenerative cycling by decomposers;
- decrease biomass by reduction of abundant species or disruption of the processes of ecosystems;
- increase biomass by removing important consumers allowing runaway production of other species.

All of these may involve changes in production and lowered human usefulness of the system. These are examples; additional effects can occur. The specific impacts of pollution at a site can be determined only through long-term study of that portion of the ocean and the changes that occur.

It is clear that man, through his numbers and his actions, is having increasingly pronounced effects on organisms, populations, and entire ecosystems. Many people willingly accept the consequences of advanced technology that are markedly deleterious, but most people become alarmed when an entire large ecosystem undergoes transformation. When society recognizes that catastrophe threatens due to its carelessness, it seeks to rearrange its demands on such ecosystems in ways that can be accommodated within the inherent capacities of the system. To provide adequate answers we need understanding of ecosystems, since knowledge at the species and population levels, however defined, will be too limited in scope to answer the questions that arise at the more highly organized level of the ecosystem.

The study of the effects of pollution on ecosystems may be undertaken by considering pollution as an additional stress on the mechanisms that keep ecosystems organized. Unless the living parts of an ecosystem are already under stress, the early effects of the introduction of toxic pollutants may contribute to the extinction of particularly susceptible species leaving the more resistant forms in a less diverse community. In communities already under stress, relatively low levels of pollution may cause the disruption of the communities.

Estuaries and intertidal regions are naturally exposed to stressful conditions. In the estuaries the ebb and flow of the tide and the fluctuating freshwater flow create changes in salinity on various time scales ranging from hourly to seasonally. In the intertidal zone the normal inhabitants are exposed to air during part of each tidal cycle. They are also subjected to vigorous wave actions on exposed beaches and headlands. Unique assemblages of organisms have evolved which manage to survive these rigorous conditions if waters remain unpolluted.

Pollutants are commonly released into such aquatic ecosystems of high natural variation in their nonliving components, and the rate of pollutant discharge usually varies from time to time. The immediate effect of these conditions is that at any fixed point in the habitat the concentration of a pollutant varies markedly with time, [illegible] not in such a way that a community can adapt itself to these variations. The result is that short-lived opportunistic species are likely to be favored in areas subject to variable aquatic pollution.

Any single toxicant may be equally virulent towards long-lived or short-lived species in the normal aquatic community. Except at outfalls where toxicants reach lethal concentrations, as in continuous discharges in stable environments, toxicants act discontinuously through time. Where water mass instabilities are such that poisonous concentrations occur on the average of once a week, for instance, it is possible for organisms with much shorter life spans to flourish briefly with large population fluctuations. Where they occur once a month, a community may evolve rapidly through a successional sequence involving a few longer-lived organisms before the next toxic concentration occurs. Where lethal dosages are as infrequent as once a year, the succession may go to the stage of some fish of medium life span, particularly if access to the area is relatively free. Because of the fluctuations with time, the community nearest an outfall is most primitive from a successional viewpoint, and as distance from the outfall increases, there is a successional gradient toward the usual climax community of an unpolluted environment.

Evaluation of the effects of pollution or of other environmental changes on the ecosystem involves studies of biological production, species diversity, energy flow, and cycling of materials. The process may be complicated by massive imports and exports at any one site. Although pathways of energy flow and efficiencies are not yet completely understood, they offer a unifying approach to these problems such as proposed by Odum (1967,[12] 1971[13]).

Species diversity is a useful attribute of biological systems. Diversity is affected by a number of factors as evidenced by the papers presented at a symposium on Diversity and Stability in Ecological Systems (Brookhaven National Laboratory 1969),[10] as well as other symposia (American Society of Civil Engineering and Stanford University 1967,[9] Olson and Burgess 1967,[14] NAS-NRC Committee on Oceanography 1970,[11] Royal Society of London 1971[15]). Some success has been achieved in the use of diversity measurements, however, and their potential for future use is high. (See the discussion of Community Structure in Section III on Freshwater Aquatic Life and Wildlife and in Appendix II-B.)

There are potentials for managing additions to coastal ecosystems in ways that benefit human uses. These are as yet poorly understood, and efforts to utilize waste heat, nutrients and other possible resources are primitive. Such possibilities merit vigorous exploration and, eventually, careful application.

FISHERIES

Major marine and coastal fisheries are based upon the capture of wild crops produced in estuaries, coastal waters, and oceans. The quantity and quality of the available supply of useful species are controlled by the nature and efficiency of the several ecosystems upon which each species depends for its life cycle. Shad, for instance, depend upon freshwater areas at the head of estuaries for spawning and for survival as eggs and larvae, open estuaries for the nutrition of juveniles, and large open coastal regions for growth and maturation. As do many other species, shad migrate over large distances. Serious pollution at any point in the lower river, the estuary, or the inshore ocean might, therefore, break the necessary patterns and reduce the fishery.

Estuaries have exceptional usefulness in support of fisheries. At least three quarters of the species in the commercial and recreational fisheries of the nation are dependent upon the estuarine ecosystem at one or more stages of their life history. Estuaries are used as obligatory spawning grounds, nursery areas, havens from parasites and predators, and as rich sources of food because of high productivity.

American fisheries exploit several levels of the coastal ecosystem. We do not utilize the plants, the producers, directly as food or in commerce except for a comparatively small harvest of kelp and other seaweeds. The primary consumers, however, are extensively utilized. These include oysters, clams, mussels, and vast quantities of filter-feeding fish such as sardines, anchovies, menhaden, and herring. Second and third level consumers, which are less abundant but frequently more desired than plankton feeders, include most of our sports fish and major commercial species such as tuna, striped bass, cod, halibut, and sea trout, as well as squid, sharks, and other species which hold potential for increased future use.

Pollutants can be detrimental to fisheries by reducing desired species through direct mortality from toxicity, smothering, intolerable heat, or other killing changes. Reduction may also occur when a pollutant has a sublethal stressing effect that significantly interferes with feeding, movement, reproduction, or some other essential function. Pollution has an indirect deleterious effect when it increases predators or parasites, reduces food organisms or essential consorts, or damages the efficiency of the ecosystem functions pertinent to the species in question. Consideration of all these occurrences must enter into efforts to protect and enhance fisheries.

Pollutants also damage marine organisms by imparting characteristics that make them unacceptable for commercial or recreational use. Economic loss has resulted from flesh tainting of fish and shellfish by oil, phenolics, and other materials affecting taste, flavor, or appearance. DDT and other persistent organics, applied on land, have accumulated in fish to levels that exceed established standards for acceptable human food. Heavy metals, e.g., mercury, can reach levels in fish several thousand times the concentration in the ambient water, destroying the economic value of the organisms involved.

More than 90 per cent of the American commercial catch and virtually all of the sport fish are taken from the estuaries and continental shelf. The total yield is difficult to estimate, involving as it does migratory species, catches by both foreign and domestic vessels, and recreational fisheries which are only partially measured. Stroud (1971)[26] estimated that the estuarine-dependent fishery of the Atlantic coast yields 535 pounds per acre of estuary for a total annual yield of 6.6×10^9 lbs. He concludes that shrinking of estuaries by filling or other destruction would reduce the yield by a directly proportional quantity. Further, he predicts that reduction of the productivity of estuaries by pollution would also produce a proportional decrease in fish production. The U.S. commercial fisheries of largest volume, in order of decreasing harvest, include menhaden, salmon, shrimp, crabs, herring, and oysters (Riley 1971).[24] The most valuable commercial harvests include shrimp, salmon, lobsters, crabs, menhaden, oysters, clams, flounders, and scallops (Riley 1971).[24]

The estuaries, as recipients of wastes both from rivers entering them and cities and industries along their shores, are obviously more immediately susceptible to pollution damage than any other part of the marine system (Clark 1967,[15] American Society of Civil Engineering and Stanford University 1967,[16] U.S. Dept. of Interior 1969,[17] and U.S. Dept. of Interior, Fish and Wildlife Service 1970[18]). Although the vulnerability of such inshore bodies of water to physical and chemical damage is exceptional, the open waters along the coast are also subject to damage from the use of these waters for waste disposal. Approximately 250 waste disposal sites are in use along the coast of the United States, and 48 million tons of wastes are estimated to have been dumped in 1968 (Council on Environmental Quality 1970).[19] These dumped wastes included dredge spoils, industrial wastes, sewage sludge, construction and demolition debris, solid wastes, and explosives (see pp. 278–283 of this Section for a more extended discussion of dumped wastes). Increased populations and technological concentration along the coasts, with simultaneous resistance to the use of land, rivers, and estuaries for disposal has stimulated proposals to increase the use of oceanic areas as receivers of wastes.

The effects of such coastal disposal on fisheries are not yet clearly established. Bechtel Corp. (1969)[17] has suggested that continued expansion of waste disposal along the Atlantic coast at the present rate of increase may, in about 30 years, significantly reduce the quality of water over the continental shelf by increased suspended solids, phosphate or nitrate enrichment, oxygen demand, heavy metals, or simultaneous effects from all of these. Preliminary studies of the effects of dumping of sewage sludge and dredging spoils from the metropolitan New York area indicate that an area of about 20 square miles has been impoverished by reduction of normal benthic populations; and indirect effects may be far more extensive (Pearce 1970).[18]

More general approaches to disposal of wastes in ocean waters have been presented by Foyn (1965)[19] Olson and Burgess (1967)[20], NAS-NRC Committee on Oceanography (1970)[21] and the Royal Society of London (1971).[22] Some discernible and disturbing changes in coastal waters are documented that prove the urgent need for better understanding of pollution effects at the edge of the oceans. The limitation that must be placed upon any such releases must be learned and put to use quickly, and we should proceed carefully while we are learning.

Fisheries provide useful indications of the biological health and productivity of marine waters. Continuous high yield of a harvestable crop of indigenous fish or shellfish free of toxicants or pathogens is an indication that water quality is satisfactory, that the environmental conditions are favorable for the total biological community, and that no contaminant is present in sufficient quantity to destroy major components of the ecosystem. Fisheries production statistics can thus serve as a sensitive indicator of environmental quanity.

Specific criteria for categories of pollutants will be given in subsequent parts of this Section. The general requirements for water quality in relation to successful fisheries include:

- favorable, not merely tolerable, environmental conditions at every location which is required in the life history of each species: this places special value on water quality of estuaries which are obligate environments for many species during at least some portion of their life cycles;
- freedom from tainting substances or conditions where useful species exist, including elements and compounds which can be accumulated by organisms to unacceptable levels;
- absence of toxic conditions or substances wherever useful species occur at any time in their life history;
- absence of sublethal deleterious conditions which reduce survival and reproductive success:
- water sufficient to maintain the health of the biological systems which support useful species;
- absence of environmental conditions which are exceptionally favorable to parasites, predators, and competitors of useful species.

MARINE AQUACULTURE

Although often considered a new approach to the world food problem, aquaculture is an ancient practice in many parts of the world. In the Orient, aquatic organisms have been successfully cultivated for centuries, usually with rather primitive and empirical techniques, but nevertheless with impressive success.

The annual world production of food through aquaculture has recently been estimated at over four million metric tons, about 6.5 per cent of the total world fish landings. Although this is derived largely from fresh water, and open-ocean maraculture is in its infancy, an unknown but significant fraction of the production is brackish-water organisms taken from estuarine systems. The distinction between freshwater and marine aquaculture is quite artificial. Because the principles, techniques, potentials, and environmental requirements for growing organisms in either fresh or salt water are much the same, the distinction is also unnecessary for the purposes of the present discussion, except as noted below.

It is difficult to assess the potential yield from marine aquaculture, dependent as it is on a primitive art undergoing rapid technological development. The introduction of present methods into new, undeveloped parts of the world could at least double the present harvest within the next decade. Judging from the experience in agriculture and terrestrial animal husbandry, much greater increase in yields should presumably be possible with advances in such fields as genetic selection and control, nutrition, habitat management and elimination or control of disease, predation, and competition. It is not inconceivable that the yield from aquaculture might one day surpass that from the harvest of wild, untended stocks of aquatic organisms. Further, since only the most desirable species are selected for aquaculture, both the economic and nutritional value per pound of cultivated organisms greatly exceeds that of the average fishery product. In the United States, expanded recent interest in coastal aquaculture will hopefully produce new techniques, products, and quantities, although economic feasibility has been difficult to achieve thus far.

Although no firm distinction can be drawn, it is convenient to think of most forms of marine aquaculture in one of two categories that will be referred to here as *extensive* and *intensive* culture. In extensive culture, animals are reared at relatively low densities in large impoundments, embayments, or sections of estuaries, either natural or man-made. The impoundments may be closed off or open to the sea, depending upon the desired degree of control, but even those that are enclosed must be located near a source of seawater so that the water may be exchanged frequently to prevent stagnation and to regulate such factors as temperature and salinity. Such exchanges are accomplished by tidal action or by pumping.

The cultivated animals may be stocked or may consist of natural populations that enter the system as larvae or juveniles. They are usually not fed but subsist on natural foods that grow in the area or are carried in with the outside seawater.

Extensive aquaculture systems are most common in the undeveloped parts of the world (e.g., Southeast Asia) where large areas of coastal mangrove swamps, marshes, and estuaries are available and are not presently in use or demand for other purposes. For example, it has been estimated that there are over six million acres of mangrove swamps in Indonesia alone that would be suitable for some form of fish farming.

In such coastal impoundments, milkfish, mullet, shrimp, and other free-swimming species are grown. In the more open situations such as embayments and arms of estuaries, non-fugitive organisms are cultivated. The oldest and most highly-developed form of marine aquaculture practiced in the United States and Europe, that of oyster culture, falls into this category. Seaweed culture in Japan and China is another interesting example of this general approach to aquaculture.

Yields from extensive aquaculture range from a few hundred pounds to, at best, about one ton per acre per year. Little, in some cases almost no, capital investment is required, and it is not a labor-intensive form of enterprise. One or two unskilled laborers can manage 100 acres or more of shrimp or milkfish ponds in Malayasia or the Philippines except during stocking and harvesting operations. This is normally a highly profitable form of business to the culturist and, despite the modest yields, extensive aquaculture is capable of making a significant contribution to the protein nutrition of many of the undeveloped parts of the world.

Intensive aquaculture makes use of flowing-water systems using flumes or raceways and is best typified by trout and salmon hatcheries that have been operated successfully in the United States and Europe for many years and have now reached a relatively high level of technical sophistication. Although originally designed to produce fish to be stocked in natural waters to enhance commercial or sports fishing, such systems are now being increasingly used for the production of fish to be marketed directly as food. Such systems were originally developed and used exclusively for rearing freshwater species, but they are now also finding application in saltwater areas for the production of marine or anadromous species.

A variation of the raceway system of intensive aquaculture is that of floating cage culture in which the animals are held in nets suspended by a floating wooden framework. These may be moored in estuaries or other protected arms of the sea, where they are exposed to strong tidal currents.

A common feature of the various kinds of intensive aquaculture is that the animals are grown closely packed at extremely high densities and depend upon the flow of large volumes of water over and around them to provide oxygen and carry away wastes. When feasible, the animals are fed artifically on prepared, pelletized food. The entire system must be carefully controlled and monitored.

Intensive aquaculture systems for the commercial production of food are in an early stage of development and have yet to prove themselves as profitable and reliable for marine species. Rapid progress is being made in this area, however, particularly in highly developed parts of the world where technological skill is available, where coastal marine areas are scarce and in high demand, and where the price of luxury seafoods is escalating. Various species of molluscs, crustaceans, and finfish are now being grown in this way, and many more are likely candidates as soom as fundamental aspects of their life history and nutrition are mastered.

The yield from intensive aquaculture per unit of area in which the organisms are grown is ecologically meaningless (as is that from a cattle feed-lot, for example) but amounts to as much as hundreds of tons per acre. More realistically, the yield from such systems may be expressed per cubic foot per minute of water flowing through it, which is usually the limiting factor.

In contrast to extensive aquaculture, intensive systems usually require high capital outlay and have a relatively high labor demand. Profits or losses are determined by small differences in the costs of food, labor, marketing, and the demand for the product.

Both extensive and intensive forms of aquaculture are heavily dependent on high quality water to sustain them. Neither is independent of the adjacent coastal marine environment. Extensive pond culture may be semi-autonomous, but as explained above, the water must be occasionally and sometimes frequently exchanged. Intensive aquaculture systems are vitally dependent on a continuous large supply of new seawater. Because of the large investment and, at best, small margin of profit, and because of the dense populations of animals maintained at any one time, intensive aquaculture represents a far greater risk.

Freshwater aquaculture systems, if strategically located near an adequate source of underground water, may be largely independent of man's activities and relatively free from the threat of pollution. This, unfortunately, is never quite true of marine aquaculture. The contiguous oceans of the world circulate freely, as do the substances man adds to them. While water movements may be predicted on large geographical and time scales, they are quite unpredictable on a local and short-term basis. An embayment or estuary whose shores are uninhabited and which may suffer no ill effects from the surrounding land may suddenly become infused with materials added to the water hundreds of miles distant and carried to the scene by winds, tides, and coastal currents. In this sense, marine aquaculture is not only more vulnerable to change than freshwater culture, but the dangers are also far less predictable.

Application of Water Quality to Aquaculture

The various toxic or otherwise harmful wastes that man adds to the coastal marine environment affect cultivated organisms much the same as they do the natural populations of the same species. These are discussed in detail elsewhere and need not be repeated here. In general the deleterious effects of wastes on organisms that are used as food by man are: (1) to kill, injure, or interfere with the growth or other vital functions of the organisms, or (2) to become concentrated in the organisms to such an extent as to render them unfit for human consumption by exceeding public health standards or by making them unpalatable. In the latter case, this may occur with no apparent accompanying impairment of the organism.

Certain aspects of aquaculture, particularly the intensive forms of culture described above, are particularly sensitive and vulnerable to various kinds of pollution—more so than their freeliving counterparts in nature. These are enumerated and discussed briefly below.

- The carrying capacity of intensive aquaculture systems is based on the flow of water and its supply of oxygen. If the concentration of oxygen in the water suddenly decreases due to an organic overload, a temperature increase, or other external causes, it may be inadequate to support the cultivated animals.
- Captive organisms cannot avoid localized unfavorable conditions (e.g., oxygen, temperature, turbidity) as can free-swimming natural populations.
- Many organisms can tolerate alterations in their environment if they are allowed to adapt and become acclimated to such changes slowly. Cultivated organisms may be, and often are subjected to sudden changes in water quality and cannot endure the initial shock, while the free-swimming natural populations can enter an affected area slowly and cautiously and allow themselves to adapt to the altered conditions.
- Cultivated organisms, particularly in the densely-crowded conditions of intensive aquaculture, may be and perhaps always are under rather severe physiological stress. Artificial diets are often incomplete or otherwise unbalanced. Unnaturally crowded living conditions may cause hormonal or other biochemical imbalance. The animals may already suffer the effects of poor water quality from their own pollutants. They are therefore particularly susceptable and vulnerable to any additional deterioration in water quality that may increase their stress condition.
- Disease is a spectre that perpetually haunts the aquaculturist. Virtually impossible to avoid or eliminate in any open system, it is usually, at best, held in check. Again, the additional stress caused by a deterioration in water quality, while not fatal in itself, may lower the resistance of the cultivated animals to epidemic disease.
- Artificially-fed cultivated organisms may be no less susceptible to accumulation of wastes, although intensively cultivated organisms that are fed entirely on an artificial diet would appear to have one advantage over natural populations of the same animals living in polluted waters. Many toxic substances such as chlorinated hydrocarbons may reach toxic or unacceptable levels in larger organisms because of concentration and amplification at each successive step in the food chain that ultimately supports the animal in question. However, there is increasing evidence that these substances also enter fishes and other organisms directly from solution in the water, across respiratory or digestive membranes. Such direct absorption of toxic material may in some cases exceed the quantities ingested and assimilated with food.

Therefore, the general recommendations for the quality of water for use in culture include: (1) continuously adequate control of those materials and conditions which are required for good health and efficient production of the cultured species; (2) absence of deleterious chemical and physical conditions, even for short or intermittent periods; (3) environmental stability; and (4) prevention of introduction of diseases that attack the organisms under culture. The specific requirements for each culture effort must be with reference to the species involved, the densities desired, and the operational design of the culture system.

MARINE WILDLIFE

Marine wildlife for the purposes of this Section is defined as those species of mammals, birds, and reptiles which inhabit estuaries or coastal and marine waters for at least a portion of their life span. The fish, invertebrates, and plankton that constitute the food webs upon which these species depend are not, therefore, considered to be wildlife in this context. The recommendations for marine wildlife, however, necessarily include all recommendations formulated to protect the fish, invertebrate, and plant communities, because wildlife can be adequately protected only if the diversity and integrity of food webs are maintained. Moreover, the recommendations must protect wildlife from pollutants that are relatively persistent in the environment, transported by wind or water currents, and concentrated or recycled in the food webs. Because of trophic accumulation, birds and mammals that occupy the higher trophic levels in the food web may acquire body burdens of toxicants that are lethal or that have significant sublethal effects on reproductive capacity, even though the concentrations of these substances in the water remain extremely low. Pollutants of concern or of potential concern are the radionuclides, heavy metals, chlorinated hydrocarbons, and other synthetic chemicals that are relatively resistant to biological and chemical degradation.

Recommendations to protect wildlife dependent upon freshwater ecosystems may in general also apply to estuaries. This is particularly true for protection of food and shelter for wildlife, pH, alkalinity, light penetration, settleable substances, and temperature. These are discussed in Section III on Freshwater Aquatic Life and Wildlife.

Marine and coastal waters constitute major sinks for persistent pollutants. Accumulation rates and steady-state levels are complex functions of input, rates of degradation, and rates of deposition in the sediments. As yet no research programs have measured accumulation rates of pollutants in coastal waters or determined whether steady-state concentrations have already been attained.

Current knowledge of the partition coefficients among concentrations in water, in sediments, and in tissues of representative species in food webs is at best fragmentary. It is assumed, however, in the evaluation of water quality that the distribution and concentration of gradients of a pollutant in an aqueous ecosystem satisfy thermodynamic equilibria requirements. The pollutants considered here are not essential to physiological functions, and do not require energy to maintain the concentration gradients. Thus the chlorinated hydrocarbons are concentrated in the lipid pools of organisms from ambient water but will not accumulate indefinitely. Rather, under equilibrium conditions, these pollutants will also be lost to ambient water, particulate matter, and sediments in satisfying thermodynamic requirements. Because the internal environments of birds and mammals are more isolated from the ambient environment than those of invertebrates and most fish, equilibrium concentrations of pollutants, particularly the chlorinated hydrocarbons, may be substantially higher.

Theoretically, therefore, measurements of pollutant concentrations in one component of an ecosystem are sufficient to indicate the level in the system as a whole when the partition coefficients among water, suspended particulate and organic material, sediments, lipid pools, surface films, and the atmosphere are known. The methodologies for measuring pollutant concentrations in sea water are as yet imperfect, and very few good measurements have so far been made. Consequently it is not practical at present to make recommendations for the relatively persistent organic pollutants based upon water concentrations, especially when partition coefficients are not known. Residue concentrations in fish are more easily determined and can more readily be associated with harm to bird and mammal populations that consume them. Recommendations for the toxic organic compounds that are trophically accumulated by marine wildlife are therefore based upon concentrations in fish.

It cannot be assumed that there is a level or concentration in the ecosystem as a whole of pollutants which are mutagens or teratogens that causes no effect on any of the wildlife species. The chlorinated dibenzo-p-dioxins are highly toxic to developing embryos (Verrett 1970)[71] and are contaminants in compounds prepared from chlorinated phenols, including the herbicide 2.4, 5-T (Verrett 1970)[71] and the widely used fungicide pentachlorophenol (Jensen and and Renberg 1972).[82] The closely related chlorinated dibenzofurans are contaminants in some PCB preparations (Vos and Koeman 1970,[74] Vos et al., 1970,[75] Vos *in press* 1972).[72] Embryonic mortality in birds is induced by these or other derivatives of PCB (Peakall et al., *in press* 1972,[53] Vos *in press* 1972).[72] For the present time the chlorinated dibenzofurans are included with PCB in the recommendations. When environmental mutagens and teratogens affect only relatively few individuals of a population, it is assumed that these will be eliminated by natural selection without harm to the species as a whole.

For other pollutants which affect specific enzyme systems or other physiological processes but not the genetic material or embryological development, it is assumed that there are levels in the environment of each below which all organisms are able to function without disrupting their life cycles. Manifestations of physiological effects, such as a certain amount of eggshell thinning or higher level of hormone metabolism, might be detectable in the most sensitive species. If environmental levels increase, the reproductive capacity of the most sensitive species would be affected first. The object of the recommendations presented is to maintain the steady-state concentrations of each pollutant below those levels which interfere with the life cycles of the most sensitive wildlife species. Input should not therefore be measured only in terms of concentrations of each pollutant in individual effluents, but in relation to the net contribution to the ecosystem. At the steady-state level, the net contribution would be zero, with the total input equal to the sum of degradation and permanent deposition in the sediments.

Bases For Recommendations

Recommendations based upon pollutant concentrations in fish must take into account the individual variation in residue concentration. The distribution is usually not Gaussian (Holden 1970;[81] Anderson and Fenderson 1970;[39] Risebrough et al. *in press* 1972),[44] with several individual fish in a sample frequently containing much higher residue concentrations than the majority. Fish samples should therefore consist of pooled collections. Samples as large as 100 fish may not be sufficient to determine mean concentrations of a pollutant with a precision of 10 per cent (Risebrough et al. *in press* 1972).[44] Practicality, however, frequently dictates against sample sizes of this magnitude, and samples consisting of 25 or more fish are suggested as a reasonable compromise.

Radionuclides

Recommendation

In the absence of data that would indicate that any of the radionuclides released by human activities are accumulated by wildlife species, it is recommended that the recommendations established for marine fish and invertebrates apply also to wildlife.

Heavy Metals

The results obtained during the baseline study of the International Decade of Ocean Exploration (IDOE) in 1971–72 have failed to indicate any evidence of pollution by heavy metals, including mercury and cadmium, above background levels in marine species (Goldberg 1972).[46] The results, suggested, however, local patterns of coastal contamination. The heavy metal analyses carried out to date of tissues of several species of petrels, strictly pelagic in their distribution (Anderlini et al. 1972);[38] and of coastal fish-eating species such as the Brown Pelican, *Pelecanus occidentalis*, (Connors et al. *in press* 1972a);[40] and the Common Tern, *Sterna hirundo* (Connors et al. *in press* 1972b)[41] have confirmed this conclusion.

Recommendation

In the absence of data indicating that heavy metals are present in marine wildlife in concentrations above natural levels, it is recommended that recommendations formulated to protect other marine organisms also apply to wildlife in order to provide protection in local areas.

Polychlorinated Biphenyls (PCB)

Evidence is accumulating that PCB does not contribute to the shell thinning that has been a major symptom of the reproductive failures and population declines of raptorial and fish-eating birds. Dietary PCB produced no shell thinning of eggs of Mallard Ducks (*Anas platyrhynchos*) (Heath et al. *in press* 1972),[49] nor did PCB have any effects on eggs of Ring Doves (*Streptopelia risoria*) (Peakall 1971).[56] A PCB effect could not be associated with the thinning of Brown Pelican (*Pelecanus occidentalis*) eggshells (Risebrough *in press* 1972).[62] PCB may increase susceptibility to infectious agents such as virus diseases (Friend and Trainer 1970).[44] Like other chlorinated hydrocarbons, PCB increases the activity of liver enzymes that degrade steroids, including the sex hormones (Risebrough et al. 1968;[64] Street et al. 1968).[67] The ecological significance of this phenomenon is not clear.

Because laboratory studies have indicated that PCB, with its derivatives or metabolites, causes embryonic death of birds (Vos et al. 1970;[73] Vos and Koeman 1970;[74] Vos *in press* 1972;[75] Peakall et al. *in press* 1972[59]) and because exceptionally high concentrations are occasionally found in fish-eating and raptorial species (Risebrough et al. 1968;[64] Jensen et al. 1969[50]), it is highly probable that PCB has had an adverse effect on the reproductive capacity of some species of birds that have shown population declines.

Median PCB concentrations in whole fish of eight species from Long Island Sound, obtained in 1970, were in the order of one milligram per kilogram (mg/kg) (Hays and Risebrough 1972),[47] and comparable concentrations have been reported from southern California (Risebrough 1969).[61] On the basis of the high probability that PCB in the environment has contributed to the reproductive failures of fish-eating birds, it is desirable to decrease these levels by at least a factor of two (see Section III on Freshwater Aquatic Life and Wildlife pp. 175–177).

Recommendation

It is recommended that PCB concentrations in any sample consisting of a homogenate of 25 or more whole fish of any species that is consumed by fish-eating birds and mammals, within the same size range as the fish consumed by any bird or mammal, be no greater than 0.5 mg/kg of the wet weight.

In the absence of a standardized methodology for the determination of PCB in environmental samples, it is recommended that estimates of PCB concentrations be based on the commercial Aroclor® preparation which it most closely resembles in chlorine composition. If the PCB composition should resemble a mixture of more than one Aroclor®, it should be considered a mixture for the basis of quantitation, and the PCB concentration reported should be the sum of the component Aroclor® equivalents.

DDT Compounds

DDT compounds have become widespread and locally abundant pollutants in coastal and marine environments of North America. The most abundant of these is DDE [2,2-bis(p-chlorophenyl) dicholoroethylene], a derivative of the insecticidal DDT compound, p,p′-DDT. DDE is more stable than other DDT derivatives, and very little information exists on its degradation in ecosystems. All available data suggest that it is degraded slowly. No degradation pathway has so far been shown to exist in the sea, except deposition in sediments.

Experimental studies have shown that DDE induces shell thinning of eggs of birds of several families, including Mallard Ducks (*Anas platyrhynchos*) (Heath et al. 1969),[48] American Kestrels (*Falco sparverius*) (Wiemeyer and Porter 1970),[77] Japanese Quail (*Coturnix*) (Stickel and Rhodes 1970)[66] and Ring Doves (*Streptopelia risorial*) (Peakall 1970).[57]

Studies of eggshell thinning in wild populations have reported an inverse relationship between shell thickness and concentrations of DDE in the eggs of Herring Gulls (*Larus argentatus*) (Hickey and Anderson 1968).[50] Double-crested Cormorants (*Phalacrocorax auritus*) (Anderson et al. 1969),[51] Great Blue Herons (*Ardea herodias*) (Vermeer and Reynolds 1970),[70] White Pelicans (*Pelecanus erythrorhynchos*) (Anderson et al. 1969),[51] Brown Pelicans (*Pelecanus occidentalis*) (Blus et al. 1972;[56] Risebrough *in press* 1972),[66] and Peregrines (*Falco peregrinus*) (Cade et al. 1970).[57]

Because of its position in the food webs, the Peregrine accumulates higher residues than fish-eating birds in the same ecosystem (Risebrough et al. 1968).[64] It was the first North American species to show shell thinning (Hickey and Anderson 1968).[50] It is therefore considered to be the species most sensitive to environmental residues of DDE.

The most severe cases of shell thinning documented to date have occurred in the marine ecosystem of southern California (Risebrough et al. 1970)[65] where DDT residues in fish have been in the order of 1–10 mg/kg of the whole fish (Risebrough *in press* 1972).[66] In Connecticut and Long Island, shell thinning of eggs of the Osprey (*Pandion haliaetus*) is sufficiently severe to adversely affect reproductive success; over North America, shell thinning of Osprey eggs also shows a significant negative relationship with DDE (Spitzer and Risebrough, *unpublished results*).[74] DDT residues in collections of eight species of fish from this area in 1970 ranged from 0.1 to 0.5 mg/kg of the wet weight (Hays and Risebrough 1972).[47] Evidently this level of contamination is higher than one which would permit the successful reproduction of several of the fish-eating and raptorial birds.

Recommendation

It is recommended that DDT concentrations in any sample consisting of a homogenate of 25 or more fish of any species that is consumed by fish-eating birds and mammals, within the same size range as the fish consumed by any bird or mammal, be no greater than 50 μg/kg of the wet weight. DDT residues are defined as the sum of the concentrations of p,p′-DDT, p,p′-DDD, p,p′-DDE and their ortho-para isomers.

Aldrin, Dieldrin, Endrin, and Heptachlor

Aldrin, dieldrin, endrin, and heptachlor constitute a class of closely related, highly toxic, organochlorine insecticides. Aldrin is readily converted to dieldrin in the environment, and heptachlor to a highly toxic derivative, heptachlor epoxide. Like the DDT compounds, dieldrin may be dispersed through the atmosphere (Tarrant and Tatton 1968,[68] Risebrough et al. 1968).[64] The greatest hazard of dieldrin is to fish-eating birds such as the Bald Eagle (*Haliaeetus leucocephalus*) (Mulhern et al. 1970);[58] the Common Egret (*Casmerodius albus*) (Faber et al. 1972)[43] and the Peregrine (*Falco peregrinus*) (Ratcliffe 1970),[60] which may accumulate lethal amounts from fish or birds that have not themselves been harmed.

These compounds are somewhat more soluble in water than are other chlorinated hydrocarbons such as the DDT group (Gunther et al. 1968);[48] partition coefficients between water and fish tissues can be assumed to be lower than those of the DDT compounds. Equivalent concentrations in fish would therefore indicate higher environmental levels of dieldrin, endrin, or heptachlor epoxide than of DDE or any of the other DDT compounds. Moreover, these compounds are substantially more toxic to wildlife than are other chlorinated hydrocarbon pesticides (Tucker and Crabtree 1970).[69] More conservative recommendations are therefore necessary.

Recommendation

It is recommended that the sum of the concentrations of aldrin, dieldrin, endrin, and heptachlor epoxide in any sample consisting of a homogenate of 25 or more whole fish of any species that is consumed by fish-eating birds and mammals, within the size range consumed by any bird or mammal, be no greater than 5 μg/kg of the wet weight.

Other Chlorinated Hydrocarbon Pesticides

Other chlorinated hydrocarbon insecticides include lindane, chlordane, endosulfan, methoxychlor, mirex, and toxaphene. Hexachlorobenzene is likely to have increased use as a fungicide as mercury compounds are phased out. This compound is toxic to birds and is persistent (Vos et al. 1968).[73] With the possible exception of hexachlorobenzene, recommendations that protect the invertebrate and fish life of estuaries from injudicious use of these pesticides will also protect the wildlife species. In light of the experience with DDT and dieldrin, the large scale use of a compound such as mirex can be expected to have adverse effects on wildlife populations.

Recommendation

It is recommended that the concentration of any of these chlorinated hydrocarbon insecticides, including lindane, chlordane, endosulfan, methoxychlor, mirex, and toxaphene, and of hexachlorobenzene, in any sample consisting of a homogenate of 25 or more whole fish of any species that is consumed by fish-eating birds and mammals, with the size range that is consumed by any bird or mammal, be no greater than 50 μg/kg of the wet weight.

Lead

No data was found to indicate that lead released into the atmosphere through the combustion of leaded gasolines has posed a hazard to wildlife populations or has resulted in an increase in body burdens of lead over background levels. Critical studies, however, have not yet been carried out. Ingestion of lead shot by waterfowl, which often mistake spent lead shot for seed or grit, kills many birds, and the pollution of marshes by lead shot is a serious problem.

Jordan (1952)[84] found that female waterfowl are about twice as sensitive to poisoning as males, and that toxicity varied greatly, depending on species, sex, and quantity and quality of food intake. A corn diet greatly increased the toxicity of lead. A study carried out by Bellrose (1951)[34] indicated that the incidence of lead shot in gizzards of waterfowl averaged 6.6 per cent in 18,454 ducks. Among infected ducks, 68 per cent contained only one shot in their gizzards, and only 17.7 per cent contained more than two (Jordan and Bellrose 1951).[85] The incidence of ingested shot appears to increase throughout the hunting season with a subsequent decline afterwards. Most losses of waterfowl due to ingested lead shot are in fall, winter, and early spring (Jordan 1952).[84] Different species show different propensities to ingest shot. Redhead (*Aythya americana*), Canvasback (*Aythya valisneria*) and Ringnecked Ducks (*Aythya collaris*) are prone to ingest shot, while Gadwall (*Anas strepera*), Teal (*Anus* sp.) and Shoveler (*Spatula clypeata*) show a low incidence. Ingestion of one shot does not appear to produce measurable changes in longevity, but six No. 6 shot are a lethal dose to Mallards, Pintail (*Anus acuta*) and Redheads (Wetmore 1919).[76] Cook and Trainer (1966)[42] found that four to five pellets of No. 4 lead shot were a lethal dose for Canada Geese (*Branta canadensis*). On a body weight basis, 6 to 8 mg/kg day is detrimental to Mallards (Coburn et al. 1951).[39]

Lead concentrations in livers of poisoned birds are of a comparable order of magnitude, ranging from 9 to 27 mg/kg in Canada Geese (Adler 1944),[29] 18 to 37 mg/kg in Whistling Swans (*Olor columbianus*) (Chupp and Dalke 1964)[35] and an average of 43 mg/kg in Mallards (*Anas platyrhynchos*) (Coburn et al. 1951).[39] These levels are 10 to 40 times higher than background, which is in the order of one mg/kg of the wet weight liver (Bagley and Locke 1967).[33]

Lead poisoning in waterfowl tends to occur especially in areas where a few inches of soft mud overlay a hard substrate. In marshes where waterfowl are hunted, the number of lead pellets per acre of marsh bottom is on the order of 25,000 to 30,000 per acre and is frequently higher (Bellrose 1959).[34] 30,000 pellets per acre are equivalent to 0.7 pellets per square foot.

The data examined indicate that the annual loss is between 0.7 per cent and 8.1 per cent of a population estimated to be 100 million birds. Although there is apparently no evidence that a loss of this magnitude has long-term detrimental effects on any species, it is considered unacceptable. Levels of lead shot in the more polluted marshes should therefore be reduced. The ultimate solution to this problem is the production of non-toxic shot.

Recommendation

In order to reduce the incidence of lead poisoning in freshwater and marine waterfowl, it is recommended that: non-toxic shot be used, or that no further lead shot be introduced into zones of shot deposition if lead shot concentrations exceed 1.0 shot per 4 square feet in the top two inches of sediment.

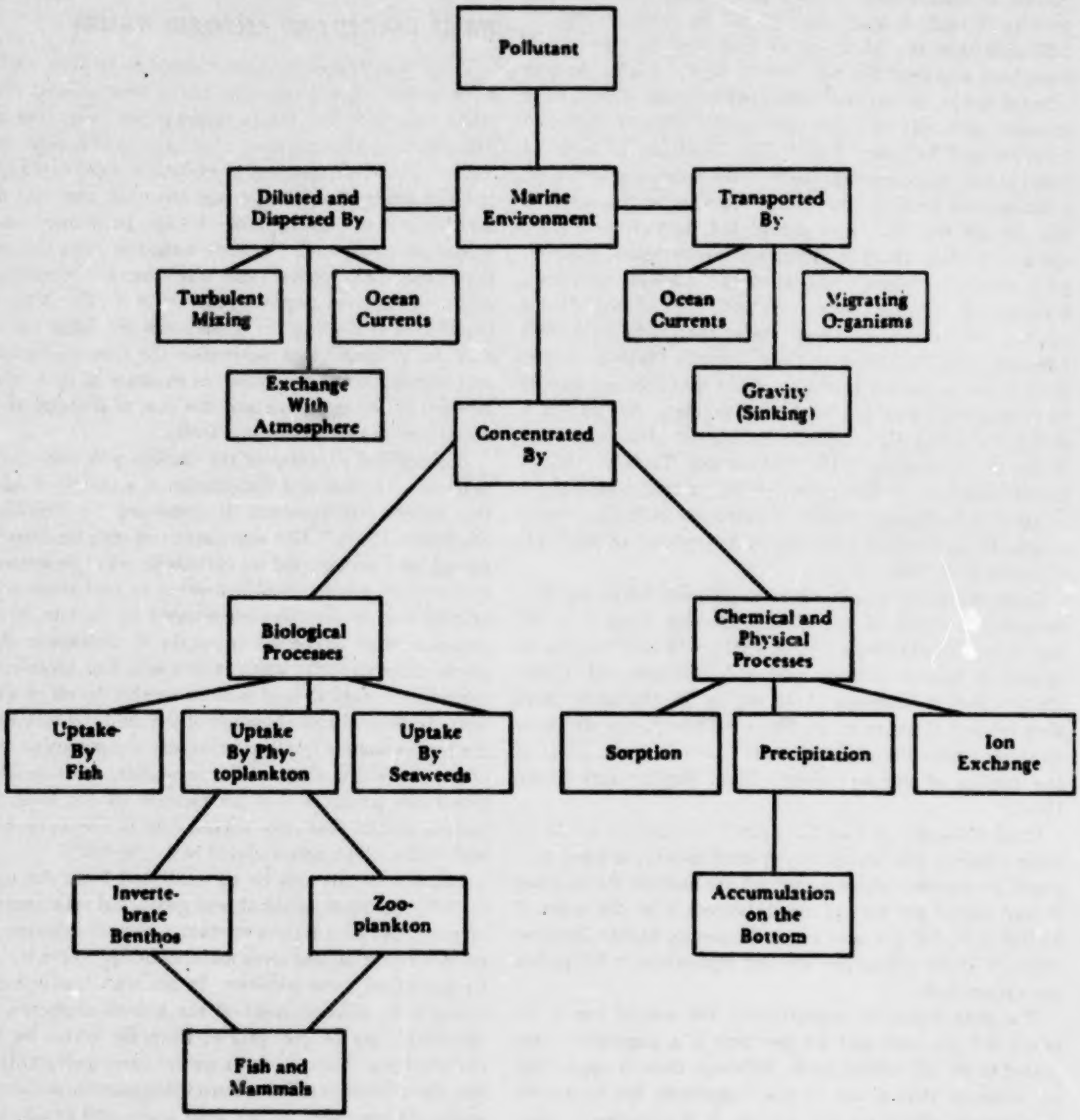
WASTE CAPACITY OF RECEIVING WATERS

When waste disposal to any natural body of water is considered, the receiving capacity of the environment must be taken into account. Waste disposal has been one of the many uses man has required of estuaries and coastal waters. These waters are capable of assimilation of definable quantities and kinds of wastes that are not toxic and that do not accumulate to unacceptable levels. In many locations wastes are being added to these waters at rates that exceed their capacity to recover; and when the rate of addition exceeds the recovery capacity, the water quality deteriorates rapidly. It is essential to understand the local conditions and the processes that determine the fate, concentration, and distribution of the pollutant in order to determine the amount of the pollutant and the rate of disposal that will not exceed the recommended levels.

A simplified diagram of the various processes that may determine the fate and distribution of a pollutant added to the marine environment is presented in Figure IV-1 (Ketchum 1967).[82] The waste material may be diluted, dispersed, and transported by various physical processes, such as turbulent mixing, ocean currents, or exchanges with the atmosphere. It may be concentrated by various biological processes, such as the direct uptake by organisms of a dissolved material in the water, and it may be transferred from organism to organism in various trophic levels of the food web. Additional concentration of the material may occur at the higher trophic levels, particularly if some organ or tissue of the body accumulates the substance, such as DDT or petroleum products that accumulate in the fatty tissues, various metals that may accumulate in the bone or liver, and iodine which accumulated in the thyroid.

Substances can also be concentrated from the environment by chemical, physical, and geological processes such as sorption. Natural waters contain a certain amount of suspended material, and some material added to the water may be sorbed on these particles. In sea water, which already contains in solution most of the known elements, added materials may be precipitated from the water by various chemical reactions. As fresh waters carry pollutants to the sea, the change in salinity causes flocculation of some of the materials suspended in the fresh water and results in their precipitation from the medium. Ion exchange reactions with the various organic compounds dissolved in sea water can also occur.

The average concentration of a given pollutant continuously added to a body of water, will tend to approach a steady state in the system. This concentration is determined by the rate of addition of the pollutant, the rate of its removal or dilution by the circulation, and the rate of its decomposition or removal by biological, chemical, or geo-


Pollutant
Diluted and Dispersed By
Marine Environment
Transported By
Turbulent Mixing
Ocean Currents
Ocean Currents
Migrating Organisms
Exchange With Atmosphere
Gravity (Sinking)
Concentrated By
Biological Processes
Chemical and Physical Processes
Uptake By Fish
Uptake By Phytoplankton
Uptake By Seaweeds
Sorption
Precipitation
Ion Exchange
Invertebrate Benthos
Zooplankton
Accumulation on the Bottom
Fish and Mammals

Ketchum 1967[82]

FIGURE IV-1—Processes That Determine the Fate and Distribution of a Pollutant Added to the Marine Environment.

logical processes. The average concentration is not always the critical concentration to be evaluated. For example, if bioaccumulation occurs, the amount accumulated in the critical organism should be evaluated, rather than the average concentration in the system as a whole. The processes of circulation and mixing may leave relatively high concentrations in one part of the system and low concentrations in another. The average conditions thus set an upper limit on what can be added to the system but do not determine the safe limit. It is clear, however, that a pollutant might be added to a body of water with vigorous circulation at a rate that could result in acceptable water quality conditions, while the same rate of addition of the pollutant to a sluggish stream might produce unacceptable levels of contamination. Thus, the characteristics of the receiving body of water must be taken into account when evaluating the effects of the pollutant upon the environment.

In a stream, the diluting capacity of a system is relatively easy to determine from the rate of addition of the pollutant and the rate of stream flow. The pollutant is carried downstream by the river flow, and "new" water is always available for the dilution of the pollutant. This is not necessarily true of lakes where the pollution added over a long period of time may accumulate, because only a small fraction of the added pollutant may be removed as a result of flushing by the outflow. In estuaries, the situation is further complicated by the mixture of salt and fresh water, because a pollutant added at a mid-point in the estuary can be carried upstream by tidal mixing just as the salt is carried upstream. The upstream distribution of a conservative pollutant is porportional to the upstream distribution of salt, whereas the downstream distribution of the pollutant is proportional to the downstream distribution of fresh water.

In either lakes or estuaries, the average retention time or the half-life of the material in the system can be used to estimate the average concentration that the pollutant will achieve in the system. In lakes, an estimate of the average retention time can be derived from the ratio of the volume of the lake divided by the rate of inflow (or outflow). When the lake is stratified, only part of the volume of the lake enters into the active circulation, and an appropriate correction must be made. In estuaries and coastal waters, a similar calculation can be made by comparing the volume of fresh water in the estuary with the rate of river inflow. The amount of fresh water in any given sample can be computed from the determination of salinity. In stratified estuaries such as a fjord, only the part of the system that is actively circulated should be taken into account. This may be adequately done by the choice of the appropriate base salinity in computing the fresh water content. Examples of the mean retention time of a few bodies of water calculated as described above are presented in Table IV-1.

Lakes with large volumes superficially appear to have a great capacity to accept waste materials. If the retention time is long, however, this merely means that it takes a long time to build up to steady-state concentration, and it will take a comparably long time to recover from a steady-state concentration once it is achieved. For Lake Superior, for example, it would take 128 years to remove half of the steady-state concentration of a pollutant that had been achieved over 185 years at the average rate of input. Aquatic environments in which the circulation is more rapid will achieve a steady-state concentration of a pollutant more quickly and will also recover more quickly.

TABLE IV-1—*Average Retention Times and Half Lives for River Water in the Great Lakes and in Various Estuaries and Coastal Regions*

	Surface area mi²	Mean retention time	Half life	Reference
Lake Superior	31,820	185 yrs.	128 yrs.	Beeton (1969)
Lake Michigan	22,400	100 yrs.	69 yrs.	Beeton (1969)
Lake Huron	23,010	30 yrs.	21 yrs.	Beeton (1969)
Lake Erie	9,930	2.6 yrs.	1.8 yrs.	Beeton (1969)
Lake Ontario	7,520	8 yrs.	5.6 yrs.	Beeton (1969)
Continental Shelf				
Cape Cod to Hatteras to 1,000 ft. contour	25,000	1.6–2.0 yrs.	1.1–1.4 yrs.	Ketchum and Keen (1955)
New York Bight	483 to 892	6.0–7.4 days	4.1–5.15 days	Ketchum et al. (1951)
Bay of Fundy	3,300	76 days	52 days	Ketchum and Keen (1953)
Delaware Bay				
high river flow	....	40–125 days	28–87 days	
low river flow				Ketchum (unpublished) (1952)
Raritan Bay				
high river flow	45	15–30 days	10–21 days	Ketchum (1951a, b)
low river flow				
Long Island Sound	930	30 days	25 days	Riley (1952)

Nonconservative pollutants are those that change with time by processes which are additional to circulation and dilution. The half-life of these substances in the environment is the product of these processes and the processes of circulation and dilution. For radioactivity, for example, the half-life is the time needed for the normal radioactive decay to dissipate half of the radiation of the material. This is different for each radioisotope and may vary from fractions of a second to centuries. The half-life for the decomposition of the organic matter in sewage in marine systems is probably on the order of days and will be dependent on temperature. The decomposition of sewage, however, releases the fertilizing elements in the organic molecule, and these will persist in the environment. In contrast to these rapid changes, the half-life of the chlorinated hydrocarbon pesticides is probably of the order of 10 years in the marine environment, though this is an estimate and not a direct determination. Heavy toxic metals, which may also pollute the environment, do not decay but persist indefinitely, though their location and forms in the system may change with time.

The greatest pollution danger arises from the addition of persistent materials to those ecosystems with slow circulations. Under these conditions, the waste concentration will increase slowly until a steady-state level is reached. If circulation is more rapid, the system will reach steady-state more quickly, but the concentration for a given rate of addition will be less. If the material is not persistent, the rate of decomposition may be more important than circulation in determining the steady-state concentration. If the products of decomposition are persistent, however, these will accumulate to levels greater than those in the original discharge. Local concentrations, such as can be found in the deeper waters of stratified systems or in trapping embayments, may be more significant than the average concentration for the whole system. In short, the recommendations cannot be used to determine the permissible amount of a pollutant to be added or a rate of addition without detailed knowledge of the specific system which is to receive the waste.

Mixing Zones

When a liquid discharge is made to a receiving system, a zone of mixing is created. In the past, these zones have frequently been approved as sites of accepted loss, exempted from the water quality standard for the receiving water. Physical description, biological assessment, and management of such zones have posed many difficult problems. The following discussion deals with criteria for assuring that no significant damage to marine aquatic life occurs in such mixing zones. Although recent public, administrative, and scientific emphasis has focused on mixing zones for the dispersion of waste heat, other uses of the mixing zone concept are also included in these considerations.

Definition of a Mixing Zone A mixing zone is a region in which an effluent is in transit from the outfall source of the receiving waters. The effluent is progressively diluted, but its concentration is higher than in the receiving waters.

Approach to the Recommendation Mixing zones must be considered on a case-by-case basis because each proposed site involves a unique set of pertinent considerations. These include the nature, quantity, and concentration of the effluent material; the physical, chemical and biological characteristics of the mixing area and receiving waters; and the desired uses of the waters. However, the following general recommendation can be established for the purpose of protecting aquatic life in areas where effluents are mixing with receiving waters:

> *The total time-toxicity exposure history must not cause deleterious effects in affected populations of important species, including the post-exposure effects.*

Meeting the Recommendation Special circumstances distinguish the mixing zone from the receiving waters. In the zone, the duration of exposure to an effluent may be quite brief, and it is usually substantially shorter than in the receiving waters, so that assays involving long periods of exposure are not as helpful in predicting damage. In addition, the concentration of effluent is higher than in receiving waters. Therefore, the development of specific requirements for a specific mixing zone must be based upon the probable duration of the exposure of organisms to the effluent as well as on the toxicity of the pollutant.

The recommendation can be met in two ways: use of a probably-safe concentration requirement for all parts of the mixing zone; or accurate determination of the real concentrations and duration of exposures for important species and good evidence that this time-toxicity exposure is not deleterious. The latter, more precise approach to meeting the recommentation will require:

- determination of the pattern of exposure of important species to the effluent in terms of time and concentration in the mixing zone;
- establishment of the summed effects on important species;
- determination that deleterious effects do not occur.

Complexities in the Marine Environment Some of the problems involved in protecting marine aquatic life are similar to those in lacustrine and fluvial fresh waters and, in general, the recommendations in Section III, pp. 112–115 are applicable to marine situations. There are, however, special complexities in evaluating mixing zones in coastal and oceanic waters. These include:

- the exceptional importance of sessile species, especially in estuaries and near shore, where effluents originate;
- the presence of almost all species in the plankton at some stage in the life history of each, so that they may be entrained in the diluting waters;
- obligate seasonal migrations by many fish and some invertebrates;
- oscillation in tidal currents, mixing mechanisms and in resulting concentrations, dilution rates, and dispersion patterns.

None of these affect the general recommendation, but they do contribute to the difficulty of applying it.

Theoretical Approach to Meeting the Recommendation Any measure of detrimental effects of a given concentration of a waste component on aquatic or marine organisms is dependent upon the time of exposure to that waste concentration, at least over some restricted but definable period of time. For a given species and substance, under a given set of environmental conditions, there will be some critical concentration below which a particular measure of detrimental effects will not be observed, regardless of the duration of exposure. Above the critical concentration, the detrimental effects will be observed if the exposure time is sufficiently long. The greater the concentration of the substance, the shorter the time of exposure to cause a specified degree of damage. The water quality characteristics for mixing zones are defined so that the organisms to be protected will be carried or move through the zone without being subjected to a time-exposure history that would produce unacceptable effects on the population of these species in the water body.

In order to quantify this statement, the following quantities are defined:

T50,C,E = time of exposure of a critical aquatic or marine species to a concentration, C, of a given pollutant, under a constant set of environmental conditions, E, which produces 50 per cent mortality of the critical species.

T0,C′,E = time of exposure of a critical aquatic or marine species to a concentration, C′, of a given pollutant, under a constant set of environmental conditions, E, which produces *no unacceptable* effects on the critical species.

For some pollutants, C and C′ for a given time of exposure may be related by:

$$C' = C - \Delta C_a$$

where ΔC_a is the amount by which the concentration which produces a 50 per cent mortality must be decreased in order that *no unacceptable* effects of the pollutant on a given critical species will occur. For example, in the case of temperature, it has been shown that at temperatures 2 C below those which produce a 50 per cent mortality, no observable detrimental effects occur. For temperature, then, 2 C is a conservative value of ΔC_a.

For other pollutants, notably chemical toxicants, C′ is related to C by the relationship:

$$C' = k \cdot C$$

where k is the ratio of the concentration at which *no unacceptable* effects occur to the concentration which produces a 50 per cent mortality with both concentrations determined over the same exposure time.

It is difficult to establish with statistical confidence a relationship between T0, C′, E and C′, for a large number of species, by direct laboratory experiments. However, laboratory experiments can be used to determine, for the critical species of the receiving waterbody, the relationship between pollutant concentration and the time period of exposure necessary to produce a 50 per cent mortality. Thus, it is necessary to obtain, by experiment, the form and constants of a function of the pollutant concentration, $f_1(C)$, such that

$$T50,\ C,\ E = f_1(C).$$

Conservative estimates of ΔC_a or of k can be obtained dependent upon decisions as to acceptable effects from additional laboratory studies. Once ΔC_a or k have been established, the relationship $C' = C - \Delta C_a$, or the relationships $C' = k \cdot C$, depending on the properties of the particular waste materials, can be combined with the above equation relating T50, C, E and C, to produce an equation relating T0, C′, E and C′. That is:

$$T0,\ C',\ E = f_2(C').$$

This equation gives the maximum time that a particular species could be exposed to a concentration C′ without resulting in unacceptable effects on the population of this species. The water quality recommendations for the mixing zone are satisfied if, for any organisms carried through the mixing zone with the flow or purposefully moving through the zone, the time of exposure satisfies the relationship

$$1 \geqslant \frac{\text{time of exposure}}{f_2(C')}$$

where C′ is the concentration of a specified pollutant in the mixing zone.

Because, in fact, the concentration in the mixing zone decreases with distance from the point of discharge, and hence organisms carried through the plume will be subjected to concentrations which are continually decreasing with time, a more suitable quantitative statement of water quality characteristics necessary for the mixing zone is:

$$1 \geqslant \frac{\Delta T1}{f_2(C'_1)} + \frac{\Delta T2}{f_2(C'_2)} + \frac{\Delta T3}{f_2(C'_3)} \cdots \frac{\Delta Tn}{f_2(C'_n)}$$

where the time of exposure of an organism passing through the mixing zone has been broken into n increments, ΔT1, ΔT2, ΔT3, etc. long. The organism is considered to be exposed to concentration C'_1 during the time interval ΔT1, to concentration C'_2 during the time interval ΔT2, etc. The sum of the individual ratios must then not exceed unity.

The above theory is applied in the recommendations and examples in Section III on Freshwater Aquatic Life and Wildlife, pp. 112–115, and in the Freshwater Appendix II-A, pp. 403–407.

METHODS OF ASSESSMENT

It is the purpose of this discussion to explain the approaches considered in deriving the recommendations given in this Section. Because the biological effects of a pollutant are manifest in a variety of ways, the specific technique to be used in estimating biological impact must be tailored to each specific problem. For example, acute or lethal toxicity of a given pollutant to a marine species can be evaluated by short-term bioassay in the laboratory designed to determine the concentration of the material which is lethal to half of the selected population in a fixed period of time, commonly four days (LC30-96 hours). The "safe" limit will be much lower than the concentration derived in such a bioassay, and appropriate safety factors must be applied. The safe limit should permit reproduction, growth, and all normal life processes in the natural habitat.

When a pollutant is discharged to the environment at a safe concentration determined in this way, the living organisms are exposed to a chronic, sublethal concentration. Some stages of the life cycle of the species to be protected, such as the eggs or larvae, may be more sensitive than the adult stages. It is sometimes possible to identify the critical life stage which can then be used in a bioassay. Long-term bioassays covering a substantial part of the life cycle of the organism can be conducted in the laboratory to determine chronic sublethal effects of pollutants. Various processes of the organism, such as respiration, photosynthesis, or activity may be used to evaluate sublethal effects. Some longterm chronic effects may be more subtle and more difficult to evaluate under laboratory conditions. Examples of this type include changes in breeding or migratory behavior or the development of a general debility making the organisms more suceptible to disease, predation, or to environmental stresses.

A pollutant in the marine environment may also have an effect on the ecosystem not directly associated with its effect on an individual species. Ecosystem interactions are difficult to assess in the laboratory, and techniques for evaluating them in the field are not completely satisfactory. Such interactions must be considered, however, in applying recommendations to any specific situation.

ACUTE TOXICITIES—BIOASSAYS

Detailed methods for laboratory bioassays are described in Section III, Freshwater Aquatic Life and Wildlife, and can serve as guidlines for application to the marine system. The ability to extrapolate from results of bioassay tests is limited, and the need for safety factors in their application to the environment must be emphasized. The methodologies discussed are illustrative and should be considered as guidelines for meaningful bioassays.

The most important uses of bioassays for evaluating water quality are:

- analysis of the concentration of a specific material in natural waters by means of a biological response;
- detection of toxic substances in organisms used as food for man;
- analysis of the suitability of natural waters for the support of a given species or ecosystem;
- determination of critical toxic levels of substances to selected species;
- evaluation of bio-stimulation effects by materials such as nutrients.

These purposes fall into two general categories: bioanalysis and bioresponse.

BIOANALYSIS

Bioanalysis has been used for many years to measure effects of substances on organisms. These assays may give quantitative measurements, such as weight per volume, or be expressed in arbitrary units defined by the degree of response. They are most valuable when the organism responds to a lower concentration than can be detected by available chemical or physical techniques. Such bioassays require carefully controlled procedures, and organisms and experimental conditions must be standardized. Responses are used that have been shown to have a correlation with the amount of test substance present. Preparation of test materials is rigidly controlled to avoid problems arising from synergists or antagonists administered with the test material. This is difficult and often impossible in the bioassay of materials obtained from the environment.

Bioanalysis has potential in measuring pollutants in materials to be discharged to the environment. For toxic materials, the amount of material relative to the biomass of the test organism must usually be controlled, because most toxicants exhibit a threshold effect. It is usual to determine the concentration of material at which some fraction of the maximal effect (commonly 50 per cent) occurs in a population of known and constant biomass. The fact that far lower concentrations present for a longer time might ultimately produce the same effect does not invalidate this type of assay, because quantitation is obtained by comparison with standard curves. It should, however, be realized that in the presence of detoxification mechanisms, the assay should be conducted for a period of time at which the desired effect (such as 50 per cent inhibition) occurs at the lowest possible concentration.

In assays of materials for which an organism has a natural or induced requirement, it must first be established that of all substances which could be present in the sample, only one can produce the response measured. Second, no substances present should reduce the availability of the material. If the first of these conditions is satisfied, the second can often be approached using a "system of adds" in which a graded series of concentrations of standard material are added to the unknown amount of material in the sample. The intercept of the response curve with the concentration axis is a measure of the amount present in the sample.

If zero response is at a finite concentration, a biologically effective threshold concentration (zero) must be used which has been derived from a separate experimental series in the same medium devoid of unknown amounts of test material.

BIORESPONSE

Bioassays which measure the biological *effect* of a substance or mixture on a single organism or artificial ecosystem can be used to establish water quality criteria, to monitor compliance with standards stated in terms of biological effect, or to measure the relative effects of various materials. Natural processes of equilibration, chemical degradation, and physical adsorption are specifically desired, because it is the biological *effect* rather than the *amount* of test material that is of concern. The observed effect will be determined by the availability of the material, the rate of formation or degradation, and the effect of chemical by-products; and by alterations of the environment caused by addition of the material. Whether conducted in the laboratory or in the field, this type of bioassay is performed on time scales varying from determinations of acute toxicity (commonly 96 hours or less) through determinations of incipient LC50 levels (Sprague 1969,[94] 1971[96]), and on time scales which include multiple generation chronic exposures. Each of these has its own utility and limitations.

Short-term determinations of TLm or TL50 values are primarily of value in comparing toxicities of a number of formulations which have similar modes of action. They are also useful in determining the dilution to be employed in long-term, flow-through exposure and in comparing sensitivities of various life stages of the same organism. In practical terms, each life stage must be considered a physiologically distinct organism with its own particular environmental requirements: immature stages commonly have quite a different habitat and may have different sensitivity.

It has been common practice to use information from acute toxicity studies to establish concentrations tolerable for natural waters. This is done by multiplying the level found in the bioassay by some more or less arbitrary "application factor" (Henderson 1957,[91] Tarzwell 1962[97]). Recently, there have been attempts to establish the application factor experimentally (Mount 1968,[92] Brungs 1969[90]). Application factors are discussed in Section III, Freshwater Aquatic Life and Wildlife, and that discussion is applicable to the marine system. If, in the process of conducting these assays, organisms are periodically removed to an uncontaminated medium, the time of exposure which the organism can withstand and still survive, should it escape the pollutant or should the pollutant degrade rapidly after a single addition, can also be estimated.

Determination of incipient LC50 is a valid measurement of acute toxicity, because the assay is continued until maximum effect is observed at any given concentration (Sprague 1969,[94] 1970,[95] 1971[96]). These bioassays must be conducted under conditions of continuous flow, because the degree of response cannot be limited by the absolute amount of toxicant available in the system or by the relationship between biomass and absolute amount. In practice, the technique is most applicable to compounds which reach equilibrium rapidly. Otherwise, it takes a long time to achieve maximum effect at low toxicant levels. Here, too, application factors are needed to use data from bioassayed concentrations in estimating levels for environmental protection. Theoretically, application factors account for variations in sensitivity between the life stage tested and that life stage or developmental period during which the organism is most sensitive to the compound or conditions. Application factors should also safely permit a range of naturally-occurring environmental variations that would increase sensitivity.

Long-term bioassay, in which the organism is kept through at least one complete life cycle under conditions of continuous-flow exposure, is perhaps the closest but most conservative laboratory approach to estimating environmental hazards. Where a chemical or physical attraction occurs or where the population is sessile or restricted by hydrographic features, continuous exposure to freshly added material will be a realistic model. However, where the organism might escape in nature, such a captive exposure will be unrealistic. The experimental conditions chosen may either be held constant or varied to approximate local natural changes or intermittent discharges to be expected. Adequate modelling of a particular environmental circumstance often requires varying degrees of delay between the time of test material addition and exposure of the organisms.

Duration of chronic toxicity studies is determined by the life span and reproductive cycle of the organism chosen. Micro-organisms have relatively short life cycles but may require several generations to deplete metabolite reserves and show maximum response. A greater variety of measurements can be used in long-term than in short-term testing. This variety, together with the longer period available for response and the certainty of testing the most sensitive life stage, serves to increase both the sensitivity and relevance of such tests. Differences in sensitivity between species, that may be evident in short-term tests, tend to narrow as the tests approach a full life cycle.

The maintenance of a resident population of sensitive organisms in an effluent stream or portion of a natural stream receiving effluent, can create a long-term flow-through bioassay. This technique is primarily useful as a verification of safety based on other estimates, but because the response time may be long, the results are of little use where rapid feedback of information is essential.

DESIGN OF BIOASSAYS

The bioassay system may be compartmentalized for purposes of design into (1) the substance to be tested, (2) the environment into which it will be introduced, (3) the organism(s) which will be exposed to the resultant system, and (4) the observations to be made. Each affects and is affected by the others.

The chemical and physical nature of the material to be tested has a bearing on the way it will distribute in nature and in the test system—and thus on which organisms will encounter it and in what form it will be. For example, a pure substance, highly soluble in water, may be tested for its effect directly on organisms inhabiting the water column. A material which precipitates rapidly may be readily available to organisms which ingest the precipitate and resolubilize it under conditions prevailing in the digestive tract. Materials which are only slightly soluble are often readily available to micro-organisms which have a high surface-area-to-volume ratio and are capable of taking up some substances at exceedingly low (10^{-5} to 10^{-10} M) concentrations. A highly hydrophobic material which is readily adsorbed to sediments or detritus may appear in free solution to only a limited extent or for a short time and exert a prolonged direct effect mainly on those organisms which inhabit sediments or which process sediments or detritus for food. Valid interpretation of bioassay results requires sensitive and highly specific analytical chemistry as part of the procedure. Results obtained for any bioassay organism are subject to question if anomalous behavior of the substance tested or the organisms used are subsequently established.

The organism for bioassay should be chosen on the basis of the relationship of its life stages to the various toxicant compartments and information desired. Organisms will be useful if they are readily available and can be reared and propagated in the laboratory. The size of the organisms in relation to available facilities will in part dictate a choice. All too often, these have been the primary if not the only considerations. There is a temptation to give priority to organisms that are available from standard sources with a known genetic line or from a single clone. This approach is essential when using bioassay as an analytical tool. However, it is a distinct liability when performing measurements of biological effect in natural environmental situations. Such organisms have necessarily undergone selection for traits that favor survival in artificial environments with no selective advantage given to the capacity to adapt to alterations in those environments. Furthermore, physiologically distinct races often develop in nature in response to characteristics of different localities. Maintenance of laboratory stocks may be necessary, but these stocks should be frequently renewed from fresh isolates representing the gene pool and enzymatic adaptations of the inhabitants of the particular water mass to which recommendations are to be applied.

The organisms used should be drawn from those that are most sensitive or respond most quickly to the substance or condition being tested. Bioassays of various life stages of these sensitive organisms are desirable. It is especially important that life stages to be tested include those that will most probably encounter the test material as it is expected to be found in the environment, and that the test organisms be acclimated to the test system until the characteristics to be measured become constant.

Some of the foregoing recommendations for selection assume that the developmental biology of the test organism is known. This is not often so in marine biology. Organisms should not be excluded from consideration if their absence would leave no representatives of local species which tolerate the extremes in ranges of natural environmental stress or which fill an important ecological niche.

Once an understanding of both the test material and the bioassay organism is established, a test system usually can be designed that will permit the organism to encounter the test material under circumstances approximating those in nature. In some cases it will be necessary to go to the natural water system or to impoundments, live cars, or plastic bags in order to obtain a workable approximation of environmental exposure. Care should be taken that the physical system does not interfere with the distribution of the test material or the behavior of the organism. The system selected should reflect in all important aspects the habitat to which the test organism has become adapted. Factors of importance include feeding behavior, opportunity for diurnal behavior alterations, emergence, salinity variations, turbidity, water movement, and other factors, depending on the organisms being studied.

The response or responses to be observed during long-term testing must be carefully chosen. A prime requirement is that the response being measured bear a demonstrable and preferably quantitative relationship to the survival and productivity of the test organism or of an organism which is directly or indirectly dependent on its activities. For example, a correlation may exist between the level of a test material and the amount of an enzyme present in some tissue. This is clear evidence that the organism's pattern of energy utilization has changed, but it should be demonstrated that the change in enzyme level is correlated with or predictive of changes in growth, behavior, reproduction, quality of flesh, or some other manifestation to provide an immediately meaningful interpretation.

The degree to which a response can be reported in quantitative terms affects its usefulness. Behavior, because of a high degree of variability, is much more difficult to express numerically than growth; and growth measurements are usually disruptive of the system or destructive of the organism. A balance must be sought for each system so that enough organisms and replicate treatments can be used to assure an acceptable level of statistical confidence in the results. Considerations of equipment required, rapidity, and simplicity of measurements, the inherent (control) variability of the characteristic being measured, and possible interference with the measurement by the substance being tested must enter into the choice of measurements and their frequency.

Biological characteristics that can be measured are innumerable, but some may be singled out as being more basic than others. When a given characteristic reflects many diverse processes, it is most useful in interpreting results in terms of environmental protection. Thus, measurements of reproductive success, growth, life span, adaptation to environmental stress, feeding behavior, morphology, respiration, histology, genetic alterations, and biochemical anomalies occupy a descending scale in order of the confidence that can be placed in their interpretation. This is not to say that profound changes in the structure and function of an ecosystem cannot result from subtle, prolonged, low intensity effects on some cellular process. The elimination of important species by low intensity selective factors is no less serious than instantaneous death of those species. In a sense, it is more serious, because it is less likely to be noticed and traced to its source in time to permit recovery of the ecosystem.

SUBLETHAL EFFECTS

Many biological effects of pollution may not show up in the bioassay test for acute toxicity. This would be true if the effect were slow to develop, or if the effect were to produce a general debility that might interfere with some of the normal life functions of the organism rather than killing it directly. Long-term exposure to sublethal concentrations may be necessary to produce the effect, and evaluation of this type of action is difficult in a laboratory analysis. There are a number of ways in which pollutants might affect a given population without being lethal to the adult organism used in the test such as:

Migrations

Sublethal concentrations may interfere with the normal migration patterns of organisms. The mechanisms used for orientation and navigation by migrating organisms are not well known, but in some cases chemotaxis clearly plays an important role. For example, salmon and many other anadromous fishes have been excluded from their home streams by pollution, though it is not known whether the reason is that a chemical cue has been masked or because the general chemical environment of pollution is offensive to the fish.

Behavior

Much of the day-to-day behavior of species may also be mediated by means of chemotaxic responses. The finding and capture of food or the finding of a mate during the breeding season would be included in this category of activity. Again, any pollutant that interfered with the chemoreceptors of the organism would interfere with behavioral patterns essential to the survival of the population.

Incidence of Disease

Long-term exposure to sublethal concentrations of pollutants may make an organism more susceptible to a disease. It is also possible that some pollutants which are organic in nature may provide an environment suitable for the development of disease-producing bacteria or viruses. In such cases, even though the pollutant is not directly toxic to the adult organism, it could have a profound effect on the population of the species over a longer period of time.

Life Cycle

The larval forms of many species of organisms are much more sensitive to pollution than are the adults, which are commonly used in the bioassay. In many aquatic species millions of eggs are produced and fertilized, but only two of the larvae produced need to grow to maturity and breed in order to maintain the standing stock of the species. For these species the pre-adult mortality is enormous even under the best of natural conditions. Because of an additional stress on the developing organisms, enough individuals might fail to survive to maintain the population of the species. Interrupting any stage of the life cycle can be as disastrous for the population as would death of the adults because of acute toxicity.

CATEGORIES OF POLLUTANTS

TEMPERATURE AND HEAT

An extensive discussion of heat and temperature is presented in Section III on Freshwater Aquatic Life and Wildlife (pp. 151–171). Although we accept those recommendations concerning temperature, there are certain characteristics of the marine environment that are unique and require enumeration. Some of the characteristics of the marine environment have been discussed in the introduction to this Section showing that the range of variability is greatest in the estuary, considerably less in the coastal waters, and even less in the surface waters of the open ocean; and that conditions in the deep ocean are virtually constant. Among the most important variables shown in the changes is temperature, although salinity variations are equally important under certain conditions.

The seasonal range of temperature variations is greatest in the temperate regions and becomes less as one approaches either the tropics or the poles. In the United States, the maximum seasonal temperature variation is found in the coastal waters on the southern side of Cape Cod, Massachusetts, where in winter the water may be freezing at −2.8 C and in summer the inshore coastal waters reach temperatures of 23 C, or even 25 C over wide shoal areas. At the same latitude on the Pacific coast, the water is neither so cold in the winter nor so warm in the summer. North of Cape Cod, the water is as cold in the winter time, but it does not reach as high a summer temperature; and south of Cape Cod the waters rarely reach a freezing point in winter.

Hutchins (1947)[100] discusses these ranges of variations and illustrates how they affect geographical distribution of marine species on the Atlantic European coasts and on the east and west coasts of the United States. As is obvious from the above comments, Cape Cod is a geographical boundary in the summertime but not in winter. Because temperature can control both the breeding cycle and survival of organisms, a variety of different geographical distributions can be dominated by the temperature variations at various locations along the coast (Hutchins 1947).[100]

There is increasing pressure to site power plants in the coastal zone because of the large available supply of water for cooling purposes. In 1969 there were over 86 fossil fuel power plants in the eastern coastal zones (Sorge 1969)[101] and 32 on the west coast (Adams 1969).[99] In addition, nuclear power plants are in operation, and many more are planned for siting on the coast in the future. Provided that the temperatures are kept within the limit prescribed in the recommendations and that the recommendations for mixing zones (pp. 228–232) are complied with, these heated effluents may have no serious impact on the marine environment. However, organisms passing through the cooling system of the power plants may be killed either by the direct effect of temperature, by pressure changes in the system, or by chlorination if it is used to keep the cooling system free of attached growth.

In the tropics, disposal of waste heat in the marine environment may be impossible in the summertime. Bader and Roessler (1972)[98] discussed the temperature problems created by the power plants at Turkey Point, near Miami, Florida. Thorhaug et al. (1972)[103] showed that tropical marine organisms live precariously close to their upper thermal limit and are thus susceptible to the stress of additional thermal effluents. To abide by the temperature recommendations in tropical waters, it is generally necessary to prohibit discharge of heated effluents during the summertime.

It is clear from this and from the discussion in Section III that additional studies will be needed on the temperature tolerances of the species directly involved. Organisms from estuaries and marine waters have not been studied as extensively as have freshwater fishes, but some data are included in the tabular material in the freshwater report. On the basis of information available at this time, the marine panel finds that the recommendations in Section III, Freshwater Aquatic Life and Wildlife, appear to be valid for the estuarine and marine waters as well (see pp. 160, 161, 164, 165, and 166–171 of Section III).

INORGANIC CHEMICALS, INCLUDING HEAVY METALS AND pH

The hazardous and biologically active inorganic chemicals are a source of both local and world-wide threats to the marine environment. Certain of these chemicals may pose no immediate danger but may lead to undesirable long-term changes. Others, such as boron, may pose serious health hazards and yet have poorly understood biological effects in the marine environment. Nevertheless, they can be a significant constituent in certain waste waters and should be discussed here.

The inorganic chemicals that have been considered in this study are listed alphabetically in Table IV-2; those most significant to the protection of the marine environment are discussed below.

TABLE IV-2—Inorganic Chemicals to be Considered in Water Quality Criteria for Aquatic Life in the Marine Environment

Elements	Equilibrium species (reaction)	Natural concentration in sea water[a] μg/l	Pollution categories[b]
Aluminum	$Al(OH)_3$, solubility of Al_2O_3 approx. 200 μg/l	10	IV c
Ammonia	NH_3, NH_4^+	....	IV c
Antimony	$Sb(OH)_6^-$	0.45	IV c ?
Arsenic	As_2O_3 is oxidized to $HAsO_4^{2-}$	2.6	II c
Barium	Ba^{2+}	30	IV c
Beryllium	$Be(OH)_2$, solubility of BeO approx. 10 μg/l	0.0006	IV c ?
Bismuth	$Bi(OH)_3$, solubility of Bi_2O_3 is unknown (low)	0.02	IV c ?
Boron	$B(OH)_3$, $B(OH)_4^-$	4.6×10^3	IV c
Bromine	Br_2, HBrO, Br^-	6.7×10^4	IV c
Cadmium	$CdCl^+$, $CdCl_2$, $CdCl_3^-$ (the last two are probably the main forms)	0.02	II c
Calcium	Ca^{2+}	4.1×10^5	IV c
Chlorine	Cl_2, HClO	....	IV c
Chromium	$Cr(OH)_3$, solubility of Cr_2O_3 unknown (low)	0.04	IV c ?
Cobalt	Co^{2+}	0.4	IV c
Copper	Cu^{2+}, $CuOH^+$, $CuHCO_3^+$, $CuCO_3$ (probably main form) $CuCl^+$, complexed also by dissolved amino acids	1	IV c
Cyanide	HCN (90%), CN^- (10%)	....	III c
Fluoride	F^- (50%), MgF^+ (50%)	1300	IV c
Gold	$AuCl_2^-$	.01-2	IV c
Hydrogen Ion (Acidity)	$HCl+HCO_3^- \rightarrow H_2O+Cl^-+CO_2$ $H_2SO_4+2HCO_3^- \rightarrow 2H_2O+SO_4^{2-}+2CO_2$	pH=8 (alk=0.0024 M)	III c
Iron	$Fe(OH)_3$, solubility of FeOOH approx. 5 μg/l	10	IV c
Lead	Pb^{2+}, $PbOH^+$, $PbHCO_3^+$, $PbCO_3$, $PbSO_4$, $PbCl^+$ (probably main form)	0.03	I a
Magnesium	Mg^{2+}	1.3×10^6	IV c
Manganese	Mn^{2+}	2	IV c
Mercury	$HgCl_2$, $HgCl_3^-$, $HgCl_4^{2-}$ (main form)	0.1	I b
Molybdenum	MoO_4^{2-}	10	IV c
Nickel	Ni^{2+}	7	III c
Nitrate	NO_3^-	6.7×10^2	III c
Phosphorus	Red phosphorus reacts slowly to phosphate $H_2PO_4^-$ and HPO_4^{2-}		
Selenium	SeO_4^{2-}	0.45	III c ?
Silicon	$Si(OH)_4$, $SiO(OH)_3^-$	3×10^3	IV c
Silver	$AgCl_2^-$	0.3	III c
Sulfide	S^{2-}	....	II c
Thallium	Tl^+	0.1	III c
Titanium	$Ti(OH)_4$, solubility of TiO_2 unknown (low)	2	IV b ?
Uranium	$UO_2(CO_3)_3^{4-}$	3	III c
Vanadium	VO_2OH^-	2	IV a ?
Zinc	Zn^{2+}, $ZnOH^+$, $ZnCO_3$, $ZnCl^+$ (probably main form)	2	III c

[a] These values are approximate but they are representative for low levels in unpolluted sea water.

[b] I–IV order of decreasing measure; a—worldwide, b—regional, c—local (coastal, bays estuaries, single dumpings). ? indicates some question of the ranking as a measure and/or whether the pollutional effect is local, regional, or worldwide.

Adapted and modified from the Report of the Seminar on Methods of Detection, Measurement and Monitoring of Pollutants in the Marine Environment. Food and Agriculture Organization 1971.

Forms of Chemical and Environmental Interactions

The form in which a chemical appears in the environment depends on the chemical and physical characteristics of the element, its stability, and the characteristics of the environment in which it is found. An element that is easily reduced or oxidized will undergo rapid changes, especially in sediments that alternate between oxidized and reduced states; while an element that is highly stable, such as gold, will retain its elemental identity in virtually all environmental conditions. Most elements are found in combined states, such as ore which can be a sulfide or a complex mineral containing oxygen, silica, and sulfur.

Certain elements are released into the environment by the processing of ores. Cadmium, for example, is not found uncombined in nature to any large extent but is a commercial by-product of zinc smelting. Other metallic elements can be brought into solution by the action of bacteria. Contamination from base metals may arise in abandoned mines, where tailings or slag heaps are attacked by physical and chemical weathering processes and bacteria to allow leaching of metallic ions into receiving waters. In strip mining, sulfides are oxidized to produce sulfuric acid, which may be a pollutant in itself or help to bring certain elements into solution.

The action of bacteria also transforms metals in another way. In anaerobic sediments, bacteria can convert inorganic metallic mercury into methyl mercury compounds. Such organo-metallic complexes are highly toxic to mammals, including man.

Biological Effects

Acute toxicity data for inorganic chemical compounds under controlled laboratory conditions, as represented for example by 96-hour LC50, are presented in Appendix III, Table 1, (pp. 449–460). Because of the lack of marine data, most of the information is based on freshwater bioassay data, which provide some measure of acute toxicity for the marine environment as well.

The concentrations of elements at which sublethal, chronic effects become manifest are also important. Sublethal concentrations of pollutants can have serious consequences in estuaries where migrating anadromous fishes linger to become acclimatized to changing salinities. Although the fish may not be killed outright, the stress of the sublethal concentrations may cause biochemical and physiological deficiencies that could impair life processes of the fish, preventing migrating adults from reaching their spawning grounds or reproducing. Pippy and Hare (1969)[247] suggested that heavy metals put fish under stress and may lead to infestation by diseases. Appendix III, Table 2 (pp. 461–468), summarizes data on the sublethal chronic effects of inorganic chemicals on fish and other aquatic organisms. As in Appendix III, Table 1, information on freshwater organisms has been included because of the paucity of tests in sea water. There is a clear need for toxicological work on the sublethal effects of pollutants on marine organisms.

At low concentrations, many elements are necessary to life processes, while at higher concentrations the same elements may be toxic. The effects of long-term exposure to low levels of most chemicals, singly or in combination, are generally unknown.

Laboratory bioassays are conducted under controlled conditions usually with single chemicals. Such tests provide toxicological information that must precede studies with mixtures closer to actual conditions. These mixtures must reflect the conditions and the composition of water in specific areas of discharge, because substances are rarely isolated when found in the environment. The probabilities of synergism and antagonism are enhanced by increased complexity of effluents. Synergism and antagonism in the environment are poorly understood. Copper is more toxic in soft water than in hard water where the calcium and the magnesium salts contributing to water hardness tend to limit or antagonize copper toxicity. Arsenic renders selenium less toxic and has been added to feeds for cattle and poultry in areas high in selenium. As examples of synergism, copper is considerably more toxic in the presence of mercury, zinc, or cadmium salts (LaRoche 1972),[211] and cadmium makes zinc and cyanide more toxic. Synergism or antagonism is expected to occur more frequently in water containing numerous chemical compounds than in one with few such compounds. Therefore, a complex chemical medium such as sea water can increase the probability of synergism or antagonism when a pollutant is introduced.

The effects of pollutants can be considered in terms of their biological end points. Such irreversible effects as carcinogenesis, mutagenesis, and teratogenesis provide identifiable end points in terms of biological consequences of pollutants. The effects of substances may vary with species or with stages of the life cycle (See Methods of Assessment, p. 233).

A distinction must be made between the effects of pollutants harmful to the quality of an organism as a product for human consumption and those harmful to the organism itself. While the levels of mercury that render fish unacceptable for marketing do not, on the basis of the limited information available at this time, appear to have any adverse effect on the fish themselves, they cause condemnation of the product for human consumption. This may also be true for other elements that lend themselves to bioaccumulation. Elemental phosphorus leads to illness and eventual mortality of fish themselves (Jangaard 1970).[191] At the concentrations of phosphorus found in the liver and other vital organs, the fish may have been toxic to human beings as well. The recommendations for the elements subject to biological accumulation in the marine environment must be set at a low level to protect the organisms. There is also need to establish recommendations based on human health, and a need to protect the economic value of fisheries affected by accumulations of some of these elements.

Data on the accumulation of inorganic chemicals by aquatic organisms are given in Appendix III, Table 3 (pp. 469–480). The maximum permissible concentrations of inorganic chemicals in food and water, as prescribed by the U.S. Food and Drug Administration and by drinking water standards of various agencies, are given in Appendix III, Table 4 (pp. 481–482).

The elements essential to plant and animal nutrition in the marine environments have been included in Table IV–2. They constitute some of the ordinary nutrients, e.g., silicon and nitrate, as well as the micro-constituents, such as iron, molybdenum, and cobalt. Although it is recognized that these elements are required for algal nutrition, one must not be caught in the misconception that "if a little is good, a lot is better."

Metals

Metals reach the marine environment through a variety of routes, including natural weathering as well as municipal and industrial discharges. Metals are particularly susceptible to concentration by invertebrates. Vinogradov's (1953)[294] classic work on the accumulation of metals by organisms in the marine environment has been expanded in more recent treatises (Fukai and Meinke 1962,[166] Polikarpov 1966,[240] Bowen et al. 1971,[129] Lowman et al. 1971).[221]

Metals present in the marine environment in an assimilable form usually undergo bioaccumulation through the food chain. Thus, elements present in low concentrations in the water may be accumulated many thousandfold in certain organisms. Established maximum permissible levels of some of these metallic ions render fish unacceptable for the commercial market (U.S. Department of Health, Education, and Welfare, Food and Drug Administration 1971,[236] Kolbye 1970[206]). Food and drug control agencies must impose stringent requirements on the content of certain hazardous elements, such as mercury, which, during 1970, led to condemnation of much of the fish caught in waters of the Canadian Prairies and the southern Great Lakes. Much of the swordfish and about 25 per cent of the tuna caught by the Japanese have exceeded the maximum permissible limit (Wallace et al. 1971).[295]

Studies conducted on Atlantic salmon (*Salmo salar*) in St. Andrew's, New Brunswick, show that low concentrations of zinc and copper mixtures will set up avoidance reactions (Sprague 1965,[268] Sprague and Saunders 1963[271]). Adult salmon migrating to spawn can be diverted by low concentrations of these base metals such as those leached from mine tailings. There are indications that as much as 25 per cent of spawning salmon (*Salmo salar*) may return to sea without going through the spawning act if concentrations of zinc and copper are high enough to induce avoidance reactions (Sprague 1965).[268] There may be other similar important behavioral reactions stimulated by low concentrations of some of the metals.

In the following review of different inorganic constituents, the total amount of each element is considered in the discussion and recommendation, unless otherwise stated. Whereas some of the methods of analysis for constituents recommended for fresh water and waste water can also be used in marine environments, the interference from salt demands other specialized techniques for many elements (Strickland and Parsons 1968,[273] Food and Agriculture Organization 1971[164]).

Not only has the recent literature been reviewed in this examination of the properties and effects of inorganic constituents, but various bibliographic and other standard references have been liberally consulted (The Merck Index 1960,[225] McKee and Wolf 1963,[216] Wilber 1969,[299] NRC Committee on Oceanography 1971,[227] U.S. Department of the Interior Federal Water Pollution Control Administration 1968,[287] Canada Interdepartmental Committee on Water 1971[139]).

Alkalinity or Buffer Capacity, Carbon Dioxide, and pH

The chemistry of sea water differs from that of fresh water largely because of the presence of salts, the major constituents of which are present in sea water in constant proportion. The weak-acid salts, such as the carbonates, bicarbonates, and borates, contribute to the high buffering capacity or alkalinity of sea water. This buffering power renders many wastes of a highly acidic or alkaline nature, which are often highly toxic in fresh water, comparatively innocuous after mixing with sea water.

The complex carbon dioxide-bicarbonate-carbonate system in the sea is described in standard textbooks (Sverdrup et al. 1946,[276] Skirrow 1965[264]). Alkalinity and the hydrogen-ion concentration, as expressed by pH (Strickland and Parsons 1968),[273] are the best measure of the effects of highly acidic or highly alkaline wastes.

European Inland Fisheries Advisory Commission (1969)[134] and Kemp (1971)[202] reviewed the pH requirements of freshwater fishes. Because of the large difference in buffer capacities, techniques for measurement and definitions of alkalinity are quite different for marine and fresh waters. The normal range of pH encountered in fresh water is considerably wider than that found in sea water, and for this reason, freshwater communities are adapted to greater pH extremes than are marine communities.

Sea water normally varies in pH from surface to bottom because of the carbon dioxide-bicarbonate-carbonate equilibria. Photosynthetic and respiratory processes also contribute to variations in pH. At the sea surface, the pH normally varies from 8.0 to 8.3, depending on the partial pressure of carbon dioxide in the atmosphere and the salinity and temperature of the water. A large uptake of carbon dioxide during photosynthesis in the euphotic zone leads to high pH values exceeding 8.5 in exceptional cases. Release of carbon dioxide during decomposition in intermediate and bottom waters results in a lowering of pH. In shallow, biologically-active waters, particularly in warm tropical and subtropical areas, there is a large diurnal variation in pH with values ranging from a high of 9.5 in the daytime to a low of 7.3 at night or in the early morning.

The toxicity of most pollutants increases as the pH increases or decreases from neutral (pH 7). This is true for complex mixtures, such as pulp mill effluents (Howard and Walden 1965),[183] for constituents which dissociate at different pH (e.g., H_2S and HCN), and for heavy metals. The toxicity of certain complexes can change drastically with pH. Nickel cyanide exhibits a thousandfold increase in toxicity with a 1.5 unit decrease in pH from 8.0 to 6.5 (Robert A. Taft Sanitary Engineering Center 1953,[256] Doudoroff et al. 1966[140]). pH may also determine the degree of dissociation of salts, some of which are more toxic in the molecular form than in the ionic form. Sodium sulfide is increasingly toxic with decreasing pH as $S^{=}$ and HS^{-} ions are converted to H_2S (Jones 1948).[illegible] The tolerance of fish to low concentrations of dissolved oxygen, high temperatures, cations, and anions varies with pH. Therefore, non-injurious pH deviations and ranges depend on local conditions.

There are large fluctuations in natural pH in the marine environment. Changes in pH indicate that the buffering capacity of the sea water has been altered and the carbon dioxide equilibria have shifted. The time required for mixing of an effluent with a large volume of sea water is exceedingly important. When the pH of the receiving sea water undergoes an increase or decrease, its duration can be important to the survival of organisms. At present, there are not sufficient data with which to assign time limits to large departures of pH.

Fish tolerate moderately large pH changes in the middle of their normal pH ranges. Small pH changes at the limits of their ranges and also in the presence of some pollutants can have significant deleterious effects.

Plankton and benthic invertebrates are probably more sensitive than fish to changes in pH. Oysters appear to perform best in brackish waters when the pH is about 7.0. At a pH of 6.5 and lower, the rate of pumping decreases notably, and the time the shells remain open is reduced by 90 per cent (Loosanoff and Tommers 1948,[211] Korringa 1952[207]). Oyster larvae are impaired at a pH of 9.0 and killed at 9.1 in a few hours (Gaarder 1932).[147] The upper pH limit for crabs is 10.2 (Meinck et al. 1956).[227]

Recommendation

Changes in sea water pH should be avoided. The effects of pH alteration depend on the specific conditions. In any case, the normal range of pH in either direction should not be extended by more than 0.2 units. Within the normal range, the pH should not vary by more than 0.5 pH units. Ad-

dition of foreign material should not drop the pH below 6.5 or raise it above 8.5.

Aluminum

Aluminum, one of the most abundant elements in the earth's crust, does not occur in its elemental form in nature. It is found as a constituent in all soils, plants, and animal tissues. Aluminum is an amphoteric metal; it may be in solution as a weak acid, or it may assume the form of a flocculent hydroxide, depending on the pH. In the aluminum sulfate form (alum), it is used in water treatment as a coagulant for suspended solids, including colloidal materials and microorganisms.

Aluminum may be adsorbed on plant organisms, but very little ingested by animals is absorbed through the alimentary canal. Goldberg et al. (1971)[172] reported an aluminum concentration factor for phytoplankton (*Sargassum*) ash of 65 and for zooplankton ash of 300. However, Lowman et al. (1971),[251] in their compilation of concentration factors for various elements, noted that aluminum was reported to be concentrated 15,000 times in benthic algae, 10,000 times in plankton (phyto- and zoo-), 9,000 times in the soft parts of molluscs, 12,000 times in crustacean muscle, and 10,000 times in fish muscle.

In fresh water, the toxicity of aluminum salts varies with hardness, turbidity, and pH. Jones (1939)[196] found the lethal threshold of aluminum nitrate for stickleback (*Gasterosteus aculeatus*) in very soft water to be 0.07 mg/l. Using tap water with the same compound tested on the same species, Anderson (1948)[113] reported a toxic threshold of less than 5×10^{-6} molar aluminum chloride (1.35 mg/l Al). Average survival times of stickleback in different concentrations of aluminum in the nitrate form have been reported as one day at 0.3 mg/l and one week at 0.1 mg/l (Doudoroff and Katz 1953).[189] It was noted by the same authors that 0.27 mg/l aluminum in the nitrate form did not apparently harm young eels in 50 hours' exposure.

Because of the slightly basic nature of sea water, aluminum salts tend to precipitate in the marine environment. These salts have exhibited comparatively low toxicities with 96-hour LC50's of 17.8 mg/l for redfish tested in sea water with aluminum chloride (Pulley 1950).[283] Concentrations of 8.9 mg/l of aluminum (from $AlCl_3$) did not have a lethal effect on marine fish and oysters tested (*Cynoscion nebulosus, Sciaenops ocellatus, Fundulus grandis, Fundulus similis, Cyprindon variegatus, Ostrea virginica*) (Pulley 1950).[283] The flocs of precipitated aluminum hydroxide may affect rooted aquatics and invertebrate benthos. Wilder (1952)[300] noted no significant effect on lobsters (*Homarus americanus*) of a tank lined with an aluminum alloy (Mn, 1 to 1.5 per cent; Fe, 0.7 per cent; Si, 0.6 per cent; Cu, 0.2 per cent, and Zn, 0.1 per cent).

Aluminum hydroxide can have an adverse effect on bottom communities. Special precautions should be taken to avoid disposal of aluminum-containing wastes in water supporting commercial populations of clams, scallops, oysters, shrimps, lobsters, crabs, or bottom fishes.

Recommendation

Because aluminum tends to be concentrated by marine organisms, it is recommended that an application factor of 0.01 be applied to marine 96-hour LC50 data for the appropriate organisms most sensitive to aluminum. On the basis of data available at this time, it is suggested that concentrations of aluminum exceeding 1.5 mg/l constitute a hazard in the marine environment, and levels less than 0.2 mg/l present minimal risk of deleterious effects.

Ammonia

Most of the available information on toxicity of ammonia is for freshwater organisms. For this reason, the reader is referred to the discussion of ammonia in Section III on Freshwater Aquatic Life and Wildlife (p. 186). Because of the slightly higher alkalinity of sea water and the larger proportion of un-ionized ammonium hydroxide, ammonia may be more toxic in sea water than in fresh water (Doudoroff and Katz 1961).[181] Holland et al. (1960)[192] noted a reduction in growth and a loss of equilibrium in chinook salmon (*Oncorhynchus tshawytscha*) at concentrations 3.5 to 10 mg/l of ammonia. Dissolved oxygen and carbon dioxide decrease the toxicity of ammonia (U.K. Department of Science and Research 1961).[294] Lloyd and Orr (1969),[217] in their studies on the effect of un-ionized ammonia at a pH of 8 to 10, found 100 per cent mortality with 0.44 mg/l NH_3 in 3 hours for rainbow trout (*Salmo gairdneri*). This confirmed earlier results of 100 per cent mortality in 24 hours at 0.4 mg/l. The toxicity increased with pH between 7.0 and 8.2.

Recommendation

It is recommended that an application factor of 0.1 be applied to marine 96-hour LC50 data for the appropriate organisms most sensitive to ammonia. On the basis of freshwater data available at this time, it is suggested that concentrations of un-ionized ammonia equal to or exceeding 0.4 mg/l constitute a hazard to the marine biota, and levels less than 0.01 mg/l present minimal risk of deleterious effects.

Antimony

Antimony occurs chiefly as sulfide (stibnite) or as the oxides cervantite (Sb_2O_4) and valentinite (Sb_2O_3) and is used for alloys and other metallurgical purposes. It has also been used in a variety of medicinal preparations and in numerous industrial applications. Antimony salts are used in the fireworks, rubber, textile, ceramic, glass and paint industries.

Few of the salts of antimony have been tested on fish in bioassays, particularly in sea water. However, antimony potassium tartrate ("tartar emetic") gave a 96-hour LC50 as antimony of 20 mg/l in soft water and 12 mg/l in hard water (Tarzwell and Henderson 1956,[277] 1960[278]). Cellular division of green algae was hindered at 3.5 mg/l, and movement of *Daphnia* was retarded at 9 mg/l (Bringmann and Kuhn 1959a).[131] Antimony trichloride, used in acid solution as a mordant for patent leather and in dyeing, was examined in exploratory tests on fathead minnows (*Pimephales promelas*) and gave a 96-hour LC50 as antimony of 9 mg/l in soft water and 17 mg/l in hard water (Tarzwell and Henderson 1960).[278] Applegate et al. (1957)[114] reported that rainbow trout (*Salmo gairdneri*), bluegill sunfish (*Lepomis macrochirus*), and sea lamprey (*Pertomyzon marinus*) were unaffected by 5 mg/l of $SbCl_3$ or $SbCl_5$ in Lake Huron water at 13 C, saturated with dissolved oxygen, and pH 7.5 to 8.2. Jernejcic (1969)[183] noted that as little as 1.0 mg/l of antimony in the form of tartar emetic caused projectile vomiting in large mouth bass (*Micropterus salmoides*).

Antimony can be concentrated by various marine forms to over 300 times the amount present in sea water (Goldberg 1957,[171] Noddack and Noddack 1939[209]).

Recommendation

Because of the hazard of antimony poisoning to humans and the possible concentration of antimony by edible marine organisms, it is recommended that an application factor of 0.02 be applied to marine 96-hour LC50 data for the appropriate organisms most sensitive to antimony. On the basis of data available at this time, it is suggested that concentrations of antimony equal to or exceeding 0.2 mg/l constitute a hazard in the marine environment. There are insufficient data available at this time to recommend a level that would present minimal risk of deleterious effects.

Arsenic

Arsenic occurs in nature mostly as arsenides or pyrites. It is also found occasionally in the elemental form. Its consumption in the U.S. in 1968 amounted to 25,000 tons as AS_2O_3 (U.S. Department of the Interior, Bureau of Mines 1969).[280] Arsenic is used in the manufacture of glass, pigments, textiles, paper, metal adhesives, ceramics, linoleum, and mirrors (Sullivan 1969),[274] and its compounds are used in pesticides, wood preservatives, paints, and electrical semiconductors. Because of its poisonous action on microorganisms and lower forms of destructive aquatic organisms, it has been used in wood preservatives, paints, insecticides, and herbicides. Sodium arsentite has been used for weed control in lakes and in electrical semiconductors.

In small concentrations, arsenic is found naturally in some bodies of water. In its different forms, including its valence states, arsenic varies in toxicity. Trivalent arsenic is considerably more toxic than the pentavalent species in the inorganic form. It is acutely toxic to invertebrates and for this reason has found application in the control of *Teredo* and other woodborers in the AS^{+3} form. Arsenious trioxide (As_2O_3) has been used for control of the shipworm *Bankia setacia*. In the arsenate form (As^{+5}), it is of relatively low toxicity, *Daphnia* being just immobilized at 18 to 31 mg/l sodium arsenate, or 4.3 to 7.5 mg/l as arsenic, in Lake Erie water (Anderson 1944,[110] 1946[111]). The lethal threshold of sodium arsenate for minnows has been reported as 234 mg/l as arsenic at 16 to 20 C (Wilber 1969).[289]

Arsenic is normally present in sea water at concentrations of 2 to 3 $\mu g/l$ and tends to be accumulated by oysters and other molluscan shellfish (Sautet et al. 1964,[238] Lowman et al. 1971[201]). Wilber (1969)[289] reported concentrations of 100 mg/kg in shellfish. Arsenic is a cumulative poison and has long-term chronic effects on both aquatic organisms and on mammalian species. A succession of small doses may add up to a final lethal dose (Buchanan 1962).[133] The acute effects of arsenic and its compounds on aquatic organisms have been investigated, but little has been done on the sublethal chronic effects.

Surber and Meehan (1931)[276] found that fish-food organisms generally can withstand concentrations of approximately 1.73 mg/l of arsenious trioxide in sodium arsenite solution. Meinck et al. (1956)[207] reported that arsenic concentrations were toxic at 1.1 to 2.2 mg/l to pike perch (*Stizostedion vitreum*) in 2 days, 2.2 mg/l to bleak in 3 days, 3.1 mg/l to carp (*Cyrinus carpio*) in 4 to 6 days and to eels in 3 days, and 4.3 mg/l to crabs in 11 days.

Recommendation

Because of the tendency of arsenic to be concentrated by aquatic organisms, it is recommended that an application factor of 0.01 be applied to marine 96-hour LC50 data for the appropriate organisms most sensitive to arsenic. On the basis of freshwater and marine toxicity data available, it is suggested that concentrations of arsenic equal to or exceeding 0.05 mg/l constitute a hazard in the marine environment, and levels less than 0.01 mg/l present minimal risk of deleterious effects.

Barium

Barium comes largely from ores ($BaSO_4$, $BaCO_3$). It is being used increasingly in industry. The U.S. consumption in 1968 was 1.6 million tons, a growth of 78 per cent in 20 years (U.S. Department of the Interior, Bureau of Mines 1969).[280] Barium is used in a variety of industrial applications, including paper manufacturing, fabric printing and dyeing, and synthetic rubber production.

All water- or acid-soluble barium compounds are poisonous. However, in sea water the sulfate and carbonate present tend to precipitate barium. The concentration of barium in sea water is generally accepted at about 20 $\mu g/l$

(Goldberg et al. 1971),[172] although it has been reported as low as 6.2 μg/l (Bowen 1956).[125] Wolgemuth and Broecker (1970)[301] reported a range of 8 to 14 μg/l in the Atlantic and 8 to 31 μg/l in the Pacific, with the lower values in surface waters. Barium ions are thought to be rapidly precipitated or removed from solution by adsorption and sedimentation.

Bijan and Deschiens (1956)[123] reported that 10 to 15 mg/l of barium chloride were lethal to an aquatic plant and two species of snails. Bioassays with barium chloride showed that a 72-hour exposure to 50 mg/l harmed the nervous system of coho salmon (*Oncorhynchus kisutch*) and 158 mg/l killed 90 per cent of the test species (ORSANCO 1960).[244] Barium can be concentrated in goldfish (*Carassius auratus*) by a factor of 150 (Templeton 1958).[279] Soviet marine radioactivity studies showed accumulation of radioactive barium in organs, bones, scales, and gills of fish from the Northeast Pacific (Moiseev and Kardashev 1964[229]). Lowman et al. (1971)[221] listed a concentration factor for barium of 17,000 in phytoplankton, 900 in zooplankton, and 8 in fish muscle.

In view of the widespread use of barium, the effects of low doses of this element and its compounds on marine organisms under different environmental conditions should be determined. Disposal of barium-containing wastes into waters when precipitates could affect rooted aquatics and benthic invertebrates should be avoided.

Recommendation

Because of the apparent concentration of barium by aquatic organisms and the resultant human health hazard, it is recommended that an application factor of 0.05 be applied to marine 96-hour LC50 data for the appropriate organisms most sensitive to barium. On the basis of data available at this time, it is suggested that concentrations of barium equal to or exceeding 1.0 mg/l constitute a hazard in the marine environment, and levels less than 0.5 mg/l present minimal risk of deleterious effects.

Beryllium

Beryllium is found mainly in the mineral beryl and is almost nonexistent in natural waters. Its concentration in sea water is 6×10^{-4} μg/l. Beryllium is used in a number of manufacturing processes, in electroplating, and as a catalyst in organic chemical manufacture. It has also been used experimentally in rocket fuels and in nuclear reactors (Council on Environmental Quality 1971).[144] In 1968, the U.S. consumption of beryllium was 8,719 tons, a 500 per cent increase over 1948 (U.S. Department of the Interior, Bureau of Mines 1969).[289]

Beryllium has been shown to inhibit photosynthesis in terrestrial plants (Bollard and Butler 1966).[127] It would be of interest to know if there is any inhibition of photosynthesis by beryllium compounds in the marine environment.

Beryllium chloride and nitrate are highly soluble in water, and the sulfate is moderately so. The carbonate and hydroxide are almost insoluble in cold water. Toxicity tests gave a 96-hour LC50 for beryllium chloride of 0.15 mg/l as beryllium for fathead minnows (*Pimephales promelas*) in soft water; 15 mg/l for the same species in hard water (Tarzwell and Henderson 1960);[278] and 31.0 mg/l for *Fundulus heteroclitus* (Jackim et al. 1970).[199]

Beryllium has been reported to be concentrated 1000 times in marine plants and animals (Goldberg et al. 1971).[172]

Recommendation

In the absence of data specifically related to effects of beryllium on marine organisms, and because of its accumulation by marine organisms and its apparent toxicity to humans, it is recommended that an application factor of 0.01 be applied to marine 96-hour LC50 data for the appropriate organisms most sensitive to beryllium. On the basis of data available for hard fresh water, it is suggested that concentrations of beryllium equal to or exceeding 1.5 mg/l constitute a hazard to marine organisms, and levels less than 0.1 mg/l present minimal risk of deleterious effects.

Bismuth

Bismuth is used in the manufacture of bismuth salts, fusible alloys, electrical fuses, low-melting solders, and fusible boiler plugs, and in tempering baths for steel, in "silvering" mirrors, and in dental work. Bismuth salts are used in analytical chemical laboratories and commonly formulated in pharmaceuticals.

The concentration of bismuth in sea water is low, about 0.02 μg/l, probably because of the insolubility of its salts. It is unknown how much bismuth actually gets into the sea from man-made sources, but the quantity is probably small. The total U.S. production in 1969 as subcarbonate $(Bi_2O_2CO_3)_2 \cdot H_2O$ was 57 short tons (U.S. Department of Commerce 1971).[265]

There are no bioassay data on which to base recommendations for bismuth in the marine environment.

Boron

Boron is not found in its elemental form in nature; it normally occurs in mineral deposits as sodium borate (borax) or calcium borate (colemanite). The concentration of boron in sea water is 4.5 mg/l as one of the 8 major constituents in the form of borate. Boron has long been used in metallurgy to harden other metals. It is now being used in the elemental form as a neutron absorber in nuclear installations.

Available data on toxicity of boron to aquatic organisms are from fresh water (Wurtz 1945,[306] Turnbull et al. 1954,[281] LeClerc and Devlaminck 1955,[214] Wallen et al. 1957,[296] LeClerc 1960[215]). Boric acid at a concentration of 2000 mg/l showed no effect on one trout and one rudd (*Scardinius erythrophthalmus*); at 5000 mg/l it caused a discoloration of the skin of the trout, and at 80,000 mg/l the trout became immobile and lost its balance in a few minutes (Wurtz 1943).[297] The minimum lethal dose for minnows exposed to boric acid at 20 C for 6 hours was reported to be 18,000 to 19,000 mg/l in distilled water and 19,000 to 19,500 mg/l in hard water (LeClerc and Devlaminck 1955,[214] LeClerc 1960[215]). Testing mosquito fish (*Gambusia affinis*) at 20 to 26 C and a pH range of 5.4 to 9.1, Wallen et al. (1957)[296] established 96-hour LC50's of 5,600 mg/l for boric acid and 3,600 mg/l for sodium borate.

Since the toxicity is slightly lower in hard water than in distilled water, it is anticipated that boric acid and borates would be less toxic to marine aquatic life than to freshwater organisms. In the absence of sea water bioassay data, an estimate of 500 mg/l of boron as boric acid and 250 mg/l as sodium borate is considered hazardous to marine animals, based on freshwater data (Wallen et al. 1957).[296] Concentrations of 50 mg/l and 25 mg/l, respectively, are expected to have minimal effects on marine fauna.

An uncertainty exists concerning the effect of boron on marine vegetation. In view of harm that can be caused to terrestrial plants by boron in excess of 1 mg/l (Wilber 1969),[299] special precautions should be taken to maintain boron at normal levels near eel grass (*Zostera*), kelp (*Macrocystis*), and other seaweed beds to minimize damage to these plants.

Recommendation

On the basis of data available at this time, it is suggested that concentrations of boron equal to or exceeding 5.0 mg/l constitute a hazard in the marine environment, and levels less than 5.0 mg/l present minimal risk of deleterious effects. An application factor of 0.1 is recommended for boron compounds applied to marine 96-hour LC50 data for the appropriate organisms most sensitive to boron.

Bromine

In concentrated form, bromine is a strong oxidizing agent and will attack all metals and organic materials. It is one of the major constituents in sea water, present at about 67 mg/l in bromate, and is commercially extracted from the sea.

Bromine is used medicinally and for sterilization of swimming pools. It is also used in the preparation of dyestuffs and anti-knock compounds for gasolines. Molecular bromine may be discharged in effluents from salt works and certain chemical industries. Bromination of certain organic substances, such as phenols and amines, may impart offensive taste and make waters more toxic [illegible] organisms.

Kott et al. (1966)[[illegible]] found that *Chlorella* [illegible] exposed to 0.42 mg/l bromine for 4 days [illegible] concentration from 2,383 cells/mm² to [illegible] remained virtually unchanged at 0.18 mg/l [illegible] cells/mm² in controls compared to 2,[illegible] cells [illegible] exposed sample).

At concentrations of 10 mg/l in soft water, [illegible] killed *Daphnia magna* (Ellis 1937),[124] and at 20 mg/l in water of 18 to 23 C, goldfish (*Carassius auratus*) were killed [illegible] 1957).[201] A violent irritant response in marine fish was observed at 10 mg/l bromine, but no such action was perceived at 1 mg/l (Hiatt et al. 1953).[148]

The salts of bromine are relatively innocuous. The threshold of immobilization for *Daphnia magna* was 210 mg/l of sodium bromate ($NaBrO_3$) and 8200 mg/l of sodium bromide (NaBr) (Anderson 1946).[111]

Recommendation

It is recommended that free (molecular) bromine in the marine environment not exceed 0.1 mg/l and that ionic bromine in the form of bromate be maintained below 100 mg/l.

Cadmium

U.S. consumption of cadmium was 6,662 short tons in 1968 (U.S. Department of the Interior, Bureau of Mines 1969).[279] These quantities indicate that cadmium might be a significant pollutant.

Pure cadmium is not found in commercial quantities in nature. It is obtained as a by-product of smelting zinc. Cadmium salts in high concentrations have been found in a Missouri spring originating from a mine (up to 1.0[illegible] mg/ml cadmium) (ORSANCO 1955),[244] and up to [illegible] to 170 mg/kg of cadmium are found in superphosphate fertilizers (Athanassiadis 1969).[114] Cadmium is also present in some pesticides. It is being used in increasing amounts by industry (Council on Environmental Quality 1971).[[illegible]] Water-carrying pipes are also a source of cadmium (Schroeder 1970)[269] as is food (Nilsson 1969).[239] Cadmium is present in most drainage waters (Kroner and Kopp 19[illegible])[[illegible]] and may be contributing substantially to the cadmium present in inshore coastal waters. It is not known, however, whether man's input has resulted in higher levels of cadmium in estuarine or coastal waters. In sea water, cadmium is generally present at about 0.1 μg/l (Goldberg et al. [illegible]).[[illegible]]

Cadmium pollution resulting in the "Itai-itai" disease in the human population has been documented (Y[illegible] and Shigematsu 1970).[317] Schroeder et al. (1[illegible]) have found that oysters may concentrate cadmium from very low levels in ambient water. Cadmium concentrations in some marine plants and animals have been given by Mullin and Riley (1956).[238]

Concern exists that cadmium may enter the diet, like mercury, through seafood. Cadmium, like mercury, could conceivably form organic compounds which might be highly toxic or lead to mutagenic or teratogenic effects.

Cadmium has marked acute and chronic effects on aquatic organisms. It also acts synergistically with other metals. A 15-week LC50 of 0.1 mg/l and inhibition of shell growth for *Crassostrea virginica* (Pringle et al. 1968),[250] and a 96-hour LC50 of 0.03 mg/l cadmium in combination with 0.15 mg/l zinc for fry of chinook salmon (*Oncorhynchus tshawytscha*) (Hublou et al. 1954)[184] have been reported.

Fundulus heteroclitus exposed to 50 mg/l cadmium showed pathological changes in the intestinal tract after 1-hour exposure, in the kidney after 12 hours, and in the gill filaments and respiratory lamellae after 20 hours (Gardner and Yevich 1970).[179] Copper and zinc, when present at 1 mg/l or more, substantially increase the toxicity of cadmium (LaRoche 1972).[211]

Cadmium is concentrated by marine organisms, particularly the molluscs (e.g., *Pecten novazetlandicae*), which accumulated cadmium in the calcareous tissues and in the viscera (Brooks and Rumbsby 1965).[133] Lowman et al. (1971)[illegible] reported a concentration factor of 1000 for cadmium in fish muscle.

Cadmium levels in tissues of Ashy Petrel (*Oceanodroama homochroa*) from coastal waters of California were approximately twice as high as in tissues of Wilson's Petrel (*Oceanites oceanicus*) obtained in Antarctica, which had summered in the North Atlantic and Australian regions, respectively. Cadmium levels in tissues of the Snow Petrel (*Pelagodroma nivea*), a species which does not leave the Antarctic ice pack region, obtained at Hallett Station, Antarctica, were of the same order of magnitude as those in the Wilson's Petrel. Cadmium levels in eggs of the Common Tern (*Sterna hirundo*) from Long Island Sound were in the order of 0.2 mg/kg dry weight, not appreciably higher than those in the Antarctic Tern (*Sterna vittata*) from the Antarctic with levels in the order of 0.1 mg/kg (Anderlini et al. *in press*).[109] Cadmium pollution may therefore be significant locally in estuaries, but on the basis of these limited data, it does not appear to be a problem in more remote marine ecosystems. However, in view of the comparatively unknown effects of cadmium on the marine ecosystem, its apparent concentration by marine organisms, and the human health risk involved in consumption of cadmium-contaminated seafood, it is suggested that there be no artificial additions of cadmium to the marine environment.

Recommendation

The panel recommends that an application factor of 0.01 be applied to marine 96-hour LC50 data for appropriate organisms most sensitive to cadmium. On the basis of data available at this time, it is suggested that concentrations of cadmium equal to or exceeding 0.01 mg/l constitute a hazard in the marine environment as well as to human populations, and levels less than 0.2 μg/l present minimal risk of deleterious effects. In the presence of copper and/or zinc at 1 mg/l or more, there is evidence that the application factor for cadmium should be lower by at least one order of magnitude.

In the absence of sufficient data on the effects of cadmium upon wildlife, it is recommended that cadmium criteria for aquatic life apply also to wildlife.

Chlorine

Chlorine is generally present in the stable chloride form which constitutes about 1.9 per cent of sea water. Elemental chlorine, which is a poisonous gas at normal temperature and pressure, is produced by electrolysis of a brine solution. Among its many uses are the bleaching of pulp, paper and textiles, and the manufacture of chemicals.

Chlorine is used to kill so-called nuisance organisms that might interfere with the proper functioning of hydraulic systems. Chlorine disinfection is also used in public water supplies and in sewage effluents to insure that an acceptable degree of coliform reduction is achieved before the effluents enter various bodies of water. In all instances the intent is to eliminate undesirable levels of organisms that would degrade water uses. This goal is only partially reached, because the effect of chlorine on desirable species is a serious hazard.

When dissolved in water, chlorine completely hydrolizes to form hypochlprous acid (HOCl) or its dissociated ions; at concentrations below 1000 mg/l, no chlorine exists in solution as Cl_2. The dissociation of HOCl to H^+ and OCl^- depends on the pH: 4 per cent is dissociated at pH 6, 25 per cent at pH 7, and 97 per cent at pH 9. The undissociated form is the most toxic (Moore 1951).[238] Although free chlorine is toxic in itself to aquatic organisms, combinations of chlorine with ammonia, cyanide, and organic compounds, such as phenols and amines, may be even more toxic and can impart undesirable flavors to seafood.

Chlorine at 0.05 mg/l was the critical level for young Pacific salmon exposed for 23 days (Holland et al. 1960).[182] The lethal threshold for chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*) for 72-hour exposure was noted by these investigators to be less than 0.1 mg/l chlorine. In aerated freshwater, monochloramines were more toxic than chlorine and dichloramine more toxic than monochloramine. Studies of irritant responses of marine fishes to different chemicals (Hiatt et al. 1953)[191] showed a slight irritant activity at 1 mg/l and violent irritant activity at 10 mg/l. Oysters are sensitive to chlorine concentrations of 0.01 to 0.05 mg/l and react by reducing pumping activity. At Cl_2 concentrations of 1.0 mg/l effective pumping could not be maintained (Galtsoff 1946).[169]

Preliminary results show that at 15 C, salinity 30 parts per thousand (‰), mature copepods (*Acartia tonsa* and

TABLE IV-3—Copepod Mortality from Chlorine Exposure

Acartia Tonsa

Chlorine mg/l	Exposure time in minutes to give 50 percent mortality	Exposure time in minutes to give 100 percent mortality
1.0	220	>300
2.5	8.5	120
5.0	1.2	10.0
10.0	0.6	1.0

Eurytemona Affinis

Chlorine mg/l	Exposure time in minutes to give 50 percent mortality	Exposure time in minutes to give 100 percent mortality
2.5	33	125
5.0	3.0	30.0
10.0	2.0	8.0

Gentile (unpublished data) 1972.[illegible]

Eurytemona affinis) have great difficulty in surviving exposures to chlorine (Table IV-3).

Clendenning and North (1960)[141] noted that at 5 to 10 mg/l chlorine, the photosynthetic capacity of bottom fronds of the giant kelp (*Macrocystis pyrifera*) was reduced by 10 to 15 per cent after 2 days and 50 to 70 per cent after 5 to 7 days.

Chlorination in seawater conduits to a residual of 2.5 mg/l killed all fouling organisms tested (anemones, mussels, barnacles, Mogula, Bugula) in 5 to 8 days; but with 1.0 mg/l a few barnacles and all anemones survived 15 days' exposure (Turner et al. 1948).[282]

It should be further stressed that chlorine applications may often be accompanied by entrainments where the organisms are exposed to strong biocidal chlorine doses, intense turbulence, and heat (Gonzales et al. *unpublished* 1971).[313] Consideration should also be given to the formation of chlorinated products, such as chloramines or other pollutants, which may have far greater and more persistent toxicity than the original chlorine applications.

Recommendation

It is recommended that an application factor of 0.1 be used with 96-hour LC50 data from seawater bioassays for the most sensitive species to be protected.

However, it is suggested that free residual chlorine in sea water in excess of 0.01 mg/l can be hazardous to marine life. In the absence of data on the *in situ* production of toxic chlorinated products, it appears to be premature to advance recommendations.

Chromium

Most of the available information on toxicity of chromium is for freshwater organisms, and it is discussed in Section III, p. 180.

Chromium concentrations in seawater average about 0.04 µg/l (Food and Agriculture Organization 1971),[161] and concentration factors of 1,600 in benthic algae, 2,300 in phytoplankton, 1,900 in zooplankton, 440 in soft parts of molluscs, 100 in crustacean muscle, and 70 in fish muscle have been reported (Lowman et al. 1971).[221]

The toxicity of chromium to aquatic life will vary with valence state, form, pH, synergistic or antagonistic effects from other constituents, and the species of organism involved.

In long-term studies on the effects of heavy metals on oysters, Haydu (*unpublished data*)[311] showed that mortalities occur at concentrations of 10 to 12 µg/l chromium, with highest mortality during May, June, and July. Raymont and Shields (1964)[253] reported threshold toxicity levels of 5 mg/l chromium for small prawns (*Leander squilla*), 20 mg/l chromium in the form Na_2CrO_4 for the shore crab (*Carcinas maenus*), and 1 mg/l for the polychaete *Nereis virens*. Pringle et al. (1968)[260] showed that chromium concentrations of 0.1 and 0.2 mg/l, in the form of $K_2Cr_2O_7$, produced the same mortality with molluscs as the controls. Doudoroff and Katz (1953)[150] investigated the effect of $K_2Cr_2O_7$ on mummichogs (*Fundulus heteroclitus*) and found that they tolerated a concentration of 200 mg/l in sea water for over a week.

Holland et al. (1960)[182] reported that 31.8 mg/l of chromium as potassium chromate in sea water gave 100 per cent mortality to coho salmon (*Oncorhynchus kisutch*). Gooding (1954)[173] found that 17.8 mg/l of hexavalent chromium was toxic to the same species in sea water.

Clendenning and North (1960)[141] showed that hexavalent chromium at 5.0 mg/l chromium reduced photosynthesis in the giant kelp (*Macrocystis pyrifera*) by 50 per cent during 4 days exposure.

Recommendation

Because of the sensitivity of lower forms of aquatic life to chromium and its accumulation at all trophic levels, it is recommended that an application factor of 0.01 be applied to marine 96-hour LC50 data for the appropriate organisms most sensitive to chromium. On the basis of data available at this time, it is suggested that concentrations of chromium equal to or exceeding 0.1 mg/l constitute a hazard to the marine environment, and levels less than 0.05 mg/l present minimal risk of deleterious effects. In oyster areas, concentrations should be maintained at less than 0.01 mg/l.

Copper

Copper has been used as a pesticide for eliminating algae in water, and its salts have bactericidal properties. Copper is toxic to invertebrates and is used extensively in marine antifouling paints which release it to the water. It is also toxic to juvenile stages of salmon and other sensitive species

(Sprague 1964,[267] 1965,[268] Sigler et al. 1966,[263] Cope 1966[142]).

Copper was the fifth metal in U.S. consumption during 1968, following iron, manganese, zinc, and barium (U.S. Department of the Interior Bureau of Mines 1969).[290] Copper is used for such products as high transmission wires, containers, utensils, and currency because of its noncorroding properties.

Copper is widely distributed in nature and is present in sea water in concentrations ranging from 1 to 25 μg/l. In small amounts, copper is nonlethal to aquatic organisms; in fact, it is essential to some of the respiratory pigments in animals (Wilber 1969).[299] Copper chelated by lignin or citrate has been reported to be as effective as copper ion in controlling algae, but apparently it is not as toxic to fish (Ingols 1955).[196] Copper affected the polychaete *Nereis virens* at levels of approximately 0.1 mg/l (Raymont and Shields 1964)[253] and the shore crab (*Carcinus maenus*) at 1 to 2 mg/l (Wilber 1969).[299] Copper at concentrations of 0.06 mg/l inhibited photosynthesis of the giant kelp (*Macrocystis pyrifera*) by 30 per cent in 2 days and 70 per cent in 4 days (Clendenning and North 1960).[141]

Copper is toxic to some oysters at concentrations above 0.1 mg/l (Galtsoff 1932)[155] and lethal to oysters at 3 mg/l (Wilber 1969).[299] The American oyster (*Crassostrea virginica*) is apparently more sensitive to copper than the Japanese species (*Crassostrea gigas*) (Reish 1964).[254] The 96-hour LC50 for Japanese oysters exposed to copper has been reported as 1.9 mg/l (Fujiya 1960).[153] However, oysters exposed to concentrations as low as 0.13 mg/l turn green in about 21 days (Galtsoff 1932).[155] Although such concentrations of copper are neither lethal to the oysters nor, apparently, harmful to man, green oysters are unmarketable because of appearance. Therefore, in the vicinity of oyster grounds, the recommendation for maximum permissible concentrations of copper in the water is based on marketability, and it is recommended that copper not be introduced into areas where shellfish may be contaminated or where seaweed is harvested.

Copper acts synergistically when present with zinc (Wilber 1969),[299] zinc and cadmium (LaRoche 1972),[211] mercury (Corner and Sparrow 1956),[143] and with pentachlorophenate (Cervenka 1959).[137] Studies on sublethal effects of copper show that Atlantic salmon (*Salmo salar*) will avoid concentrations of 0.0024 mg/l in laboratory experiments (Sprague et al. 1965,[270] Saunders and Sprague 1967,[262] Sprague 1971[269]).

Copper is accumulated by marine organisms, with concentration factors of 30,000 in phytoplankton, 5,000 in the soft tissues of molluscs, and 1000 in fish muscle (Lowman et al. 1971).[221]

Bryan and Hummerstone (1971)[134] reported that the polychaete *Nereis diversicolor* shows a high takeup of copper from copper-rich sediments and develops a tolerance. Mobile predators feeding on this species could receive doses toxic to themselves or accumulate concentrations that would be toxic to higher trophic levels.

Recommendation

It is recommended that an application factor of 0.01 be applied to marine 96-hour LC50 data for the appropriate organisms most sensitive to copper. On the basis of data available at this time, it is suggested that concentrations of copper equal to or exceeding 0.05 mg/l constitute a hazard in the marine environment, and levels less than 0.01 mg/l present minimal risk of deleterious effects.

Cyanides

Most of the available information on toxicity of cyanides is for freshwater organisms, and is discussed in the Freshwater Aquatic Life and Wildlife section, p. 189.

Recommendation

As a guideline in the absence of data for marine organisms the panel recommends that an application factor of 0.1 be applied to marine 96-hour LC50 data for the appropriate organisms most sensitive to cyanide. On the basis of data available at this time it is suggested that concentrations of cyanide equal to or exceeding 0.01 mg/l constitute a hazard in the marine environment, and levels less than 0.005 mg/l present minimal risk of deleterious effects.

Fluorides

Fluorides have been brought to public attention in recent years because of their effects at low concentrations in human dental development and in prevention of decay. However, it must be remembered that fluorides at higher concentrations are poisons afflicting human and other mammalian skeletal structures with fluorosis (see Section II, p. 66).

Fluorine is the most reactive non-metal and does not occur free in nature. It is found in sedimentary rocks as fluorspar, calcium fluoride, and in igneous rocks as cryolite, sodium aluminum fluoride. Seldom found in high concentrations in natural surface waters because of their origin only in certain rocks in certain regions, fluorides may be found in detrimental concentrations in ground waters.

Fluorides are emitted to the atmosphere and into effluents from electrolytic reduction plants producing phosphorus and aluminum. They are also used for disinfection, as insecticides, as a flux for steel manufacture, for manufacture of glass and enamels, for preserving wood, and for assorted chemical purposes.

A review of fluoride in the environment (Marier and Rose 1971)[225] indicates that the concentration of unbound ionic fluoride (F^-) in sea water ranges between 0.4 and 0.7 mg/l. Approximately 50 per cent of the total seawater

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fluoride (0.77 to 1.40 mg/l) is bound as the double ion MgF^+.

Concentrations as low as 1.5 mg/l of fluoride have affected hatching of fish eggs (Ellis et al. 1946),[127] and 2.3 mg/l, introduced as sodium fluoride, was lethal to rainbow trout (*Salmo gairdneri*) at 18 C (Angelovic et al. 1961).[113] Virtually no information exists on long-term chronic effects of low concentrations of fluorides in sea water.

Recommendation

In the absence of data on the sublethal effects of fluorides in the marine environment, it is recommended that an application factor of 0.1 be applied to marine 96-hour LC50 data for the appropriate organisms most sensitive to fluoride. On the basis of data available at this time it is suggested that concentrations of fluoride equal to or exceeding 1.5 mg/l constitute a hazard in the marine environment, and levels less than 0.5 mg/l present minimal risk of deleterious effects.

Iron

Because of the widespread use of iron by man for his many industrial activities, iron is a common contaminant in the aquatic environment. Iron may enter water naturally from iron ore deposits; but iron is more often introduced from acid mine drainage, mineral processing, steel pickling, and corrosion. Iron usually occurs in the ferrous form, when it is released from processing plants or in mine drainage, but becomes rapidly oxidized to the ferric form in natural surface waters. The ferric salts form gelatinous hydroxides, agglomerate and flocculate, settling out on the bottom or becoming adsorbed on various surfaces. Depending on the pH and Eh, groundwater may contain a considerable amount of iron in solution, but well aerated waters seldom contain high, dissolved iron. In the marine environment, iron is frequently present in organic complexes and in adsorbed form on particulate matter.

Most of the investigations on biological effects of iron have been done in fresh water (Knight 1901,[214] Bandt 1948,[117] Minkina 1946,[229] Southgate 1948,[265] Lewis 1960,[215] ORSANCO 1960[245]). Deposition of iron hydroxides on spawning grounds may smother fish eggs, and the hydroxides may irritate the gills and block the respiratory channels of fishes (Southgate 1948,[265] Lewis 1960[215]). Direct toxicity of iron depends on its valence state and whether it is in solution or suspension.

Warnick and Bell (1969)[297] examined the effects of iron on mayflies, stoneflies, and caddisflies and obtained a 96-hour LC50 of 0.32 mg/l for the three insects. Dowden and Bennett (1965)[153] examined the effect of ferric chloride to *Daphnia magna* in static acute bioassays. They noted LC50's of 36, 21, and 15 mg/l for 1, 2, and 4 days, respectively.

Ferric hydroxide flocs removed the diatoms in the process of flocculation and settling, coating the bottom; and the iron precipitate coated the gills of white perch (*Morone americana*), minnows, and silversides in upper Chesapeake Bay (Olson et al. 1941).[242]

Tests on three types of fish gave a lethality threshold for iron at 0.2 mg/l (Minkina 1946)[229] and on carp at 0.9 mg/l if the pH was 5.5 or lower. Ebeling (1928)[155] found that 10 mg/l of iron caused serious injury or death to rainbow trout (*Salmo gairdneri*) in 5 minutes. La Roze (1955)[212] reported that dogfish were killed in 3 hours at 5 mg/l iron, whereas other research (National Council for Stream Improvement 1953)[236] indicated no deaths during one week at 1 to 2 mg/l.

Because of the slightly alkaline condition of sea water, much of the iron introduced to the sea precipitates. This adds a further problem of iron hydroxide flocs contaminating bottom sediments where rooted aquatics and invertebrates could be affected.

Special consideration should be given to avoiding discharge of iron-containing effluents into waters where commercially important bottom species or important food organisms dwell (e.g., oysters, clams, scallops, lobsters, crabs, shrimp, halibut, flounder, and demersal fish eggs and larvae).

Recommendation

On the basis of data available at this time, it is suggested that concentrations of iron equal to or exceeding 0.3 mg/l constitute a hazard to the marine environment, and levels less than 0.05 mg/l present minimal risk of deleterious effects.

Lead

The present rate of input of lead into the oceans is approximately ten times the rate of introduction by natural weathering, and concentrations of lead in surface sea water are greater than in deeper waters (Chow and Patterson 1966).[139] The isotope composition of the lead in surface waters and in recent precipitation is more similar to that of mined ore than to that in marine sediments (Chow 1968).[138] There are almost no data, however, that would suggest that the higher concentrations of lead in surface sea water derived from lead transported through the atmosphere have resulted in higher lead concentrations in marine wildlife. Lead concentrations in Greenland snow have been shown to be 16 times higher in 1964 than in 1904 (Murozumi et al. 1969).[235] In 1968 an estimated 1.8×10^5 tons of lead were introduced to the atmosphere as a result of the combustion of leaded gasoline (Council on Environmental Quality 1971).[144] This represents 14 per cent of the total lead consumption of the United States for that year. Lead poisoning of zoo animals in New York City was attributed to their breathing lead-contaminated air (Bazell 1971).[119]

Blood serum aldolase activity in higher animals exposed to small amounts of lead increased, although there were no overt signs or symptoms of poisoning (Yaverbaum 1963,[303] Wilber 1969[299]). Chronic lead poisoning in man is symptomatically similar to multiple sclerosis (Falkowska et al. 1964).[160] Muscular dystrophy has been reported as occurring in fishes and amphibians (Stolk 1962,[272] Wilber 1969[299]) and in view of these findings could, in fact, be unnatural.

Data are needed on the sublethal, long-term effects of lead on aquatic organisms, particularly those in sea water. Evidence of deleterious effect to freshwater fish has been reported for concentrations of lead as low as 0.1 mg/l (Jones 1938).[197]

Wilder (1952)[300] reported lobster dying in 6 to 20 days when held in lead-lined tanks. Pringle (*unpublished data*)[313] observed a 12-week LC50 of 0.5 mg/l lead and an 18-week LC50 of 0.3 mg/l lead with the oyster (*Crassostrea virginica*). There was noticeable change in gonadal and mantle tissue following 12 weeks exposure at concentrations of 0.1 to 0.2 mg/l of lead. Calabrese et al. (*unpublished data*)[310] found a 48-hour LC25 of 1.73 mg/l and an LC50 of 2.45 mg/l for oyster eggs of the same species.

North and Clendenning (1958)[241] reported that lead nitrate at 4.1 mg/l of lead showed no deleterious effect on the photosynthesis rate in kelp (*Macrocystis pyrifera*) exposed for four days. They concluded that lead is less toxic to kelp than mercury, copper, hexavalent chromium, zinc, and nickel.

Recommendation

In the absence of more definitive information on the long-term chronic effect of lead on marine organisms, it is recommended that concentrations of lead in sea water should not exceed 0.02 of the 96-hr LC50 for the most sensitive species, and that the 24-hour average concentration should not exceed 0.01 of the 96-hour LC50. On the basis of data available at this time it is suggested that concentrations of lead equal to or exceeding 0.05 mg/l constitute a hazard in the marine environment, and levels less than 0.01 mg/l present minimal risk of deleterious effects. Special effort should be made to reduce lead levels even further in oyster-growing areas.

Lead recommendations for the protection of wildlife are included in the discussion of Marine Wildlife p. 227.

Manganese

Manganese is one of the most commonly used metals in industry. It occurs widely in ores on land and in nodules in the deep sea. U.S. consumption in 1958 exceeded 2.2 million tons, a 45 per cent increase in 20 years (U.S. Department of Interior, Bureau of Mines 1969).[290] The metal is alloyed with iron to produce steel and in smaller quantities with copper for manganese bronze. Its salts are used in inks and dyes, in glass and ceramics, in matches and fireworks, for dry-cell batteries, and in the manufacture of paints and varnishes.

Manganese is often found with iron in ground waters, and it can be leached from soil and occur in drainage in high concentrations. The carbonates, oxides, and hydroxides are slightly soluble, so that manganous and manganic ions are rarely present in surface water in excess of 1 mg/l. Manganese is present in sea water at about 2 µg/l in the Mn^{+2} form, and is concentrated through biochemical processes to form manganese nodules, found mainly in the deep sea.

Manganese may have different effects on the lower trophic levels in fresh water and sea water. Concentrations of manganese above 0.005 mg/l had a toxic effect on certain algae in reservoirs (Guseva 1937,[174] 1939[175]), while 0.0005 mg/l in sea water stimulated growth and multiplication of certain phytoplankton (Harvey 1947).[178] Anderson (1944)[110] reported the threshold of immobilization of *Daphnia magna* as 0.63 mg/l of $KMnO_4$ and the threshold concentration for immobilization of *Daphnia magna* in Lake Erie water as 50 mg/l of $MnCl_2$ (Anderson 1948).[112] Bringmann and Kuhn (1959a)[131] reported the threshold effect for the same species as 50 mg/l of $MnCl_2$ as manganese in River Havel water at 23 C.

For the flatworm *Polycelis nigra*, the threshold concentration of manganese was reported as 700 mg/l as manganese chloride and 660 mg/l as manganese nitrate (Jones 1940).[199] Tests on organisms on which fish feed, i.e., crustacea, worms, and insect larvae, showed no apparent harm at 15 mg/l of manganese during a 7-day exposure (Schweiger 1957).[270] River crayfish were found to tolerate 1 mg/l (Meinck et al. 1956).[227]

The toxicity of manganese to fish depends on a number of factors which may vary from one situation to another. There is an apparent antagonistic action of manganese toward nickel toxicity for fish (Blabaum and Nichols 1956).[125] This may be true also for cobalt and manganese in combination, as noted for terrestrial plant life (Ahmed and Twyman 1953).[103]

Stickleback survived 50 mg/l manganese as manganese sulphate for 3 days, whereas eels withstood 2700 mg/l for 50 hours (Doudoroff and Katz 1953).[150] The lethal concentration of manganese for stickleback was given as 40 mg/l by Jones (1939),[198] and he noted that the toxic action was slow. The minimum lethal concentration of manganese nitrate for sticklebacks in tap water has been reported to be 40 mg/l as manganese (Anderson 1948,[112] Murdock 1953).[234]

The average survival times of stickelback in manganous nitrate solution were one week at 50 mg/l, four days at 100 mg/l, two days at 150 mg/l, and one day at 300 mg/l, all measured as manganese (Murdock 1953).[234] Young eels tolerated 1500 mg/l manganous sulphate for more than 25 hours (Doudoroff and Katz 1953).[150] Oshima (1931)[246] and Iwao (1936)[189] reported the lethal thresholds of manganous chloride and manganous sulphate for fish in Japan to be about 2400 and 1240 mg/l of manganese, respectively. They found that permanganates (Mn^{+7}) killed fish at 2.2 to 4.1 mg/l manganese in 8 to 18 hours, but this high oxidation form is quite unstable in water. Tench, carp, and trout tolerated 15 mg/l of manganese during 7 days exposure (Schweiger 1957).[261]

Manganous chloride was found to be lethal to minnows (*Fundulus*) in fresh water in six days at 12 mg/l $MnCl_2$; for the small freshwater fish Orizias, the 24 hour lethal concentration was about 7850 mg/l (Doudoroff and Katz 1953);[155] and for other fish 5500 mg/l (Oshima 1931,[246] Iwao, 1936[189]). The highest concentration tolerated by eels for 50 hours was 6300 mg/l (Doudoroff and Katz 1953).[155] Meinck et al. (1956)[227] noted the first toxic effects for fish of $MnCl_2$ at 330 mg/l, with the lethal concentration at 800 mg/l.

Only a few studies of sublethal effects of manganese on fish have been reported. Ludemann (1953)[222] noted some of the symptoms of toxicity of manganese to fish, crabs, and fish food organisms. Abou-Donia and Menzel (1967)[103] noted an effect of 1.25×10^{-4} M manganese (6.9 mg/l) on the enzyme acetylcholinesterase in shiner perch.

In studies on the uptake of radionuclides on the Pacific testing grounds of Bikini and Eniwetok, it was found that the neutron-induced isotope of manganese ^{54}Mn was concentrated by as much as 4000 in phytoplankton and 12,000 in the muscle or soft tissue of mollusks (Lowman 1960,[220] Lowman et al. 1971[221]). Goldberg et al. (1971)[172] list the concentration factor of manganese in marine plants and animals as approximately 3000.

Recommendation

In view of the evidence for concentration of manganese by marine organisms, an application factor of 0.02 of the 96 hr LC50 for the most sensitive species to be protected is recommended.

Until more complete information on acute and sublethal effects of manganese on marine organisms is available, it is suggested that concentrations of 0.1 mg/l or more of total manganese in the marine environment may constitute a hazard, and concentrations of less than 0.02 mg/l present minimal risk.

Mercury

Mercury naturally leaches from cinnabar (HgS) deposits. Man-made sources of mercury have been in plastics manufacture, where mercury oxide is used as a catalyst, chlor-alkali plants where mercury cells are used, mercurial slimacides used in the pulp and paper industry and in other forest product anti-fungal applications, seed dressings used in combatting smuts and other fungal diseases afflicting seeds, and in anti-fouling paints. An estimated 5000 tons of mercury per year are transferred from the continents to the oceans as a result of continental weathering (Klein and Goldberg 1970).[203] Global production of mercury is currently about twice as high, in the order of 9000 metric tons per year (Hammond 1971).[176] The burning of petroleum releases in the order of 1600 tons of mercury into the atmosphere per year (Bertine and Goldberg 1971).[122] A conservative estimate of the amount of mercury released per year into the global environment from the burning of coal is in the order of 3000 tons (Joensuu 1971).[195] The total amount of mercury estimated to be in the oceans is in the order of 10^8 metric tons, approximately three orders of magnitude higher than the total amount of mercury consumed in the United States since 1900. Mercury in marine organisms is, therefore, most probably of natural origin except in localized areas.

One hundred and eleven persons were reported poisoned, 41 died, and others suffered serious neurological damage as a result of eating fish and shellfish which had been contaminated with mercury discharged into Minamata Bay by a plastics manufacturing plant between 1950 and 1960 (Irukayama 1967).[187] In 1965, another poisoning incident was reported in Niigata, Japan, where 5 people died and 26 suffered irreversible neurological damage (Ui 1967).[292] In Minamata it was also found that cats eating the contaminated fish and shellfish took suicidal plunges into the sea, an uncommon occurrence with these mammals (Ui and Kitamura 1970).[293]

Metallic mercury can be converted by bacteria into methyl mercury (Jensen and Jernelöv 1969,[192] Jernelöv 1969,[194] Löfroth 1969[218]). Organometallic mercury is much more toxic than the metallic mercury and enters the food cycle through uptake by aquatic plants, lower forms of animal life, and fish (Jernelöv 1969).[194] The concentration factor of mercury in fish was reported as 3,000 and higher (Hannerz 1968,[177] Johnels and Westermark 1969[196]). A voluntary form of control was imposed in Sweden where anglers were requested not to eat more than one fish per week from a given lake to minimize human intake.

High mercury concentrations in birds and fish were reported on the Canadian prairies in 1969 (Fimreite 1970,[160] Wobeser et al. 1970,[301] Bligh 1971[126]). The source of the mercury in the birds was apparently mercurial seed dressing consumed with grain by the birds; whereas in fish, mercury came largely from emissions of a chlor-alkali plant using a mercury cell.

The Food and Drug Directorate of Canada set a level of 0.5 parts per million as the maximum permissible concentration in fish products. The 0.5 parts per million level was set as an interim guideline, not a regulation based on any known safe level for mercury (Canada Food and Drug Directorate, *personal communication*).[311] A similar guideline was adopted in the U.S. (Kolbye 1970).[206] These limits were based on the lethal concentrations found in Minamata Bay, Japan, and on the levels set by the World Health Organization (WHO) in cooperation with the Food and Agricultural Organization (FAO). The level set by WHO FAO was 0.05 ppm, based on total food (WHO 1967).[296] The concentrations which were found lethal to the Japanese consuming fish and shellfish contaminated by mercury were 10 to 50 mg kg total mercury (Birke et al. 1968).[124] The Swedish limit was 1.0 ppm of mercury in fish Berglund and Wretling 1967)[121] based on dry weight, which is equivalent to 0.2 ppm wet weight (Wallace et al. 1971).[293]

Although the emphasis has been on the effects of mercury on man, aquatic organisms can be affected by various mercury compounds. Mercury markedly alters the epithelium of skin and gills in fishes (Schweiger 1957).[261] Mercuric chloride in water containing developing eggs of *Paracentrotus lividis* brought about a severe disturbance of development at 10 μg/l (Soyer 1963).[266] A concentration of 5 μg/l retarded development markedly. These studies suggested that the threshold for harmful effects of mercuric chloride on developing eggs of *Paracentrotus* was around 2 to 3 μg/l (Soyer 1963).[266] Studies conducted on developing salmon eggs (*Oncorhynchus nerka* and *O. gorbuscha*) at the International Pacific Salmon Fisheries Commission Laboratory in Cultus Lake, B.C., showed that concentrations of mercury at levels exceeding 3 μg/l mercury derived from mercuric sulfate led to severe deformities (Servizi, *unpublished data*).[316] Studies are needed to examine the effects of those concentrations which are accumulated by fish over a longer period of time.

Ukeles (1962)[253] reported that 60 μg/l of ethyl mercury phosphate was lethal to all species of marine phytoplankton tested, and that as little as 0.1 to 0.6 μg/l of alkyl mercury introduced into sea water will inhibit photosynthesis and growth. Clendenning and North (1960)[141] reported that mercury added as mercuric chloride caused 50 per cent inactivation of photosynthesis of giant kelp (*Macrocystis pyrifera*) at 50 μg l during 4 days exposure, a 15 per cent decrease in photosynthesis at 100 μg/l in 1 day, and complete inactivation in 4 days.

Woelke (1961)[302] reported that 27 μg/l of mercury as mercuric chloride was lethal to bivalve larvae. The learning behavior of goldfish (*Carassius auratus*) was affected after two days by 3 μg l mercuric chloride (Weir and Hine 1970).[295] Trace amounts of copper increase the toxicity of mercury (Corner and Sparrow 1956).[143]

Mercury concentrations in tissues of the Ashy Petrel (*Oceanodroma homochroa*) from the coastal waters of California, the site of most of the mercury mines in the United States, are in the same order of magnitude as mercury concentrations in tissues of the Snow Petrel (*Pagodroma nivea*), which inhabits the Antarctic pack ice. Mercury concentrations in nine eggs of the Common Tern (*Sterna hirundo*) from Long Island Sound were only slightly higher than in nine eggs of the Antarctic Tern (*Sterna vittata*) from the Antarctic (Anderlini et al. *in press*).[109]

Environmental residues of mercury in Sweden, as measured by concentrations of mercury in feathers of several species of birds, rose dramatically in the years following 1940 and were attributed to alkyl-mercury compounds used as seed dressings (Berg et al. 1966).[129] This use of mercury caused the death of numbers of seed-eating birds (Borg et al. 1969),[130] but it does not necessarily contaminate aquatic ecosystems (Johnels and Westermark 1969).[196] Feathers of two species of fish-eating birds, the Osprey (*Pandion haliaetus*) and the Great-crested Grebe (*Podiceps cristatus*), have shown a gradual increase in mercury concentration since approximately 1900, paralleling the increase in industrial use of mercury in Sweden (Johnels and Westermark 1969).[196] Experimental work in Sweden has shown that when pheasants were fed wheat treated with methyl-mercury dicyandiamide, decreased hatchability of eggs was associated with mercury concentrations in the eggs from 1.3 to 2.0 mg/kg of the wet weight contents (Borg et al. 1969).[130] It has been suggested that environmental mercury may impair the reproductive capacity of bird species at the tops of food chains, such as falcons (Fimreite et al. 1970),[161] and in Finland mercury may have contributed to the decline of the Whitetailed Sea Eagle (*Haliaetus albicilla*) in regions where the species feeds upon marine fish and marine birds (Henriksson et al. 1966).[190] Conclusive evidence that mercury has impaired the reproductive capacity of any species of wildlife, however, has not yet been obtained and further research is necessary. Fish-eating birds and mammals are the species most likely to be affected because of their position at the top of the food chain.

The high natural levels of mercury in the marine environment and the significant additions due to natural weathering, as well as the documented hazard to marine aquatic life and to humans through marine foods, make it desirable to eliminate inputs of mercury to the marine environment beyond those occurring through continental weathering.

Recommendation

On the basis of data available at this time, it is suggested that concentrations of mercury equal to or exceeding 0.10 μg/l constitute a hazard in the marine environment.

In the absence of sufficient data on the effects of mercury in water upon wildlife, the recommendations established to protect aquatic life and public water supplies should also apply to protect wildlife.

Molybdenum

Molybdenum has been found to be a needed microconstituent in fresh waters for normal growth of phytoplankton (Arnon and Wessel 1953).[110] In mammals, exposure to molybdenum may interfere with vital chemical reactions (Dick and Ball 1945).[149]

Molybdenum metal is quite stable and is used in ferro-molybdenum for the manufacture of special tool steels. It is available in a number of oxide forms as well as the disulphide. Molybdic acid is used in a number of chemical applications and in make-up of glazes for ceramics.

Molybdenum has not been considered as a serious pollutant, but it is a biologically active metal. It may be an important element insofar as protection of the ecosystem is concerned because of its role in algal physiology. Certain species of algae can concentrate molybdenum by a factor up to 15 (Lackey 1959).[210] Bioassay tests in fresh water on the fathead minnow gave a 96-hour LC50 for molybdic anhydride (MoO_3) of 70 mg/l in soft water and 370 mg/l in hard water. Although molybdenum is essential for the growth of the alga *Scenedesmus*, the threshold concentration for a deleterious effect is 54 mg/l. Molybdenum concentration factors for marine species have been reported as: 8 in benthic algae; 26 in zooplankton; 60 in soft parts of molluscs; 10 in crustacean muscles; and 10 in fish muscle (Lowman et al. 1971).[221]

Recommendation

The panel recommends that the concentration of molybdenum in sea water not exceed 0.05 of the 96-hour LC50 at any time for the most sensitive species in sea water, and that the 24-hour average not exceed 0.02 of the 96-hour LC50.

Nickel

Nickel does not occur naturally in elemental form. It is present as a constituent in many ores, minerals and soils, particularly in serpentine-rock-derived soils.

Nickel is comparatively inert and is used in corrosion-resistant materials, long-lived batteries, electrical contacts, spark plugs, and electrodes. Nickel is used as a catalyst in hydrogenation of oils and other organic substances. Its salts are used for dyes in ceramic, fabric, and ink manufacturing. Nickel may enter waters from mine wastes, electroplating plants, and from atmospheric emissions.

Nickel ions are toxic, particularly to plant life, and may exhibit synergism when present with other metallic ions. Nickel salts in combination with a cyanide salt form moderately toxic cyanide complexes which, as nickel sulfate combined with sodium cyanide, gave a 48-hour LC50 of 2.5 mg/l and a 96-hour LC50 of 0.95 mg/l as CN^-, using fathead minnows (*Pimephales promelas*) at 20 C (Doudoroff 1956).[145] Alkaline conditions reduced toxicity of a nickel cyanide complex considerably, with concentrations below 100 mg/l showing no apparent toxic effect on fish.

Nickel salts can substantially inhibit the biochemical oxidation of sewage (Malaney et al. 1959).[223] In fresh waters, nickel has been reported to be less toxic to fish and river crabs than zinc, copper, and iron (Podubsky and Stedronsky 1948).[248] However, other investigators found nickel to be more toxic to fish than iron and manganese (Doudoroff and Katz 1953).[150]

Ellis (1937)[156] reported that nickelous chloride from electroplating wastes did not kill goldfish (*Carassius auratus*) at 10 mg/l during a 200-hour exposure in very soft water. Wood (1964)[304] reported that 12 mg/l of nickel ion kill fish in 1 day and 0.8 mg/l kill fish in 10 days. Doudoroff and Katz (1953)[150] reported survival of stickleback (*Gasterosteus aculeatus*) for 1 week in 1 mg/l of nickel as $Ni(NO_3)_2$.

The lethal limit of nickel to sticklebacks has been reported as 0.8 mg/l (Murdock 1953)[231] and 1.0 mg/l (Jones 1939).[198] The median lethal concentration of nickel chloride ($NiCl_2,6H_2O$) was reported as 4.8 mg/l for guppies (*Becilia reticulata*) (Shaw and Lowrance 1956).[272] Goldfish (*Carassius auratus*) were killed by nickel chloride at 4.5 mg/l as nickel in 200 hours (Rudolfs et al. 1953).[256] Tarzwell and Henderson (1960)[278] reported 96-hour LC50's for fathead minnows (*Pimephales promelas*) as 4.0 mg/l in soft water and 24 mg/l in hard water, expressed as $NiCl_2,6H_2O$. Anderson (1948)[112] reported a threshold concentration of nickel chloride for immobilization of *Daphnia* in Lake Erie water at 25 C to be less than 0.7 mg/l in 64 hours of exposure. Bringmann and Kuhn (1959a,[131] 1959b[132]) reported nickel chloride threshold concentrations as nickel of 1.5 mg/l for *Scenedesmus*, 0.1 mg/l for *Escherichia coli*, and 0.05 mg/l for *Microregma*.

Nickel is present in sea water at 5 to 7 μg/l, in marine plants at up to 3 mg/l, and in marine animals at about 0.4 mg/l.

Marine toxicity data for nickel are limited. The top minnow *Fundulus* was found to survive in concentrations of 100 mg/l Nickel from the chloride in salt water, although the same species was killed by 8.1 mg/l of the salt (3.7 mg/l Ni) in tap water (Thomas cited by Doudoroff and Katz 1953).[150] Long-term studies on oysters (Haydu *unpublished data*)[314] showed substantial mortality at a nickel concentration of 0.12 mg/l. Calabrese et al. (*unpublished data*)[310] found 1.54 mg/l of nickel to be the LC50 for eggs of the oyster (*Crassostrea virginica*).

Recommendation

It is recommended that an application factor of 0.02 be applied to 96-hour LC50 data on the most sensitive marine species to be protected. Although limited data are available on the marine environment, it is suggested that concentrations of nickel in excess of 0.1 mg/l would pose a hazard to marine organisms, and 0.002 mg/l should pose minimal risk.

Phosphorus

Phosphorus as phosphate is one of the major nutrients required for algal nutrition. In this form it is not normally toxic to aquatic organisms or to man. Phosphate in large quantities in natural waters, particularly in fresh waters, can lead to nuisance algal growths and to eutrophication. This is particularly true if there is a sufficient amount of nitrate or other nitrogen compounds to supplement the phosphate. Thus, there is a need for control of phosphate input into marine waters. See Sewage and Nutrients, p. 275, for a discussion of the effects of phosphate as a nutrient.

Phosphorus in the elemental form is particularly toxic and subject to bioaccumulation in much the same way as mercury (Ackman et al. 1970,[104] Fletcher 1971[162]). Isom (1960)[188] reported an LC50 of 0.105 mg/l at 48 hours and 0.025 mg/l at 163 hours for bluegill sunfish (*Lepomis macrochirus*) exposed to yellow phosphorus in distilled water at 25 C and pH 7.

Phosphorus poisoning of fish occurred on the coast of Newfoundland in 1969 and demonstrated what can happen when the form of an element entering the sea is unknown or at least not properly recognized (Idler 1969,[185] Jangaard 1970,[191] Mann and Sprague 1970[224]). The elemental phosphorus was released in colloidal form and remained in suspension (Addison and Ackman 1970).[105] After the release of phosphorus was initiated, red herrings began to appear. The red discoloration was caused by haemolysis, typical of phosphorus poisoning in herring (*Clupea harengus*), and elemental phosphorus was found in herring, among other fishes, collected 15 miles away (Idler 1969,[185] Jangaard 1970[191]).

Fish will concentrate phosphorus from water containing as little as one µg/l (Idler 1969).[185] In one set of experiments, a cod swimming in water containing one µg/l elemental phosphorus for 18 hours was sacrificed and the tissues analyzed. The white muscle contained about 50 µg/kg, the brown, fat tissue about 150 µg/kg, and the liver 25,000 µg/l (Idler 1969,[185] Jangaard 1970[191]). The experimental findings showed that phosphorus is quite stable in the fish tissues. Fish with concentrated phosphorus in their tissues could swim for considerable distances before succumbing. In addition to the red surface discoloration in herring, other diagnostic features of phosphorus poisoning included green discoloration of the liver and a breakdown of the epithelial lining of the lamellae of the gill (Idler 1969).[185]

A school of herring came into the harbor one and one-half months after the phosphorus plant had been closed down. These herring spawned on the wharf and rocks near the effluent pipe, and many of them turned red and died. A few days later, "red" herring were caught at the mouth of the harbor on their way out. The herring picked up phosphorus from the bottom sediments which contained high concentrations near the effluent pipeline (Ackman et al. 1970).[104] Subsequently, this area was dredged by suction pipeline, and the mud was pumped to settling and treatment ponds. No further instances of red herring were reported after the dredging operation, and the water was comparatively free of elemental phosphorus (Addison et al. 1971).[106]

Reports of red cod caught in the Placentia Bay area were investigated, and it was found that no phosphorus was present in the cod tissues. Surveys of various fishing areas in Newfoundland established that red cod are no more prevalent in Placentia Bay than in other areas. In laboratory studies, cod exposed to elemental phosphorus have not shown the red discoloration observed in herring and salmonids. However, cod do concentrate phosphorus in the muscle tissue as well as in the liver and can eventually succumb to phosphorus poisoning (Dyer et al. 1970).[151]

It was demonstrated by field investigations and laboratory experiments (Ackman et al. 1970,[104] Fletcher et al. 1970,[163] Li et al. 1970,[216] Zitko et al. 1970,[302] Fletcher 1971[162]) that elemental phosphorus accounted for the fish mortalities in Placentia Bay. This is not to say that other pollutants, such as fluorides, cyanides, and ammonia, were not present (Idler 1969).[185]

The conclusion was reached by the scientists working on the problem that elemental phosphorus in concentrations so low that they would be barely within the limits of detection are capable of being concentrated by fish. Further work is needed on the effects of very low concentrations of phosphorus on fish over extended periods. Discharge of elemental phosphorus into the sea is not recommended.

Recommendation

It is recommended that an application factor of 0.01 be applied to marine 96-hour LC50 data for the appropriate organisms most sensitive to elemental phosphorus. On the basis of data available at this time it is suggested that concentrations of elemental phosphorus equal to or exceeding 1 µg/l constitute a hazard to the marine environment.

Selenium

Selenium has been regarded as one of the dangerous chemicals reaching the aquatic environment. Selenium occurs naturally in certain pasture areas. Toxicity of selenium is sometimes counteracted by the addition of arsenic which acts as an antagonist. Selenium occurs in nature chiefly in combination with heavy metals. It exists in several forms including amorphous, colloidal, crystalline, and grey. Each physical state has different characteristics, soluble in one form, but insoluble in another. The crystalline and grey forms conduct electricity, and the conductivity is increased by light. This property makes the element suitable for photoelectric cells and other photometry uses. Selenium is also used in the manufacture of ruby glass, in wireless telegraphy and photography, in vulcanizing rubber, in insecticidal preparations, and in flameproofing electric cables. The amorphous form is used as a catalyst in determination of nitrogen and for dehydrogenation of organic compounds.

Ellis (1937)[156] showed that goldfish (*Carassius auratus*) could survive for 98 to 144 hours in soft water of pH ranging from 6.4 to 7.3 at 10 mg/l sodium selenite. Other data (ORSANCO 1950)[243] showed that 2.0 mg/l of selenium administered as sodium selenite was toxic in 8 days, affecting appetite and equilibrium, and lethal in 18 to 46 days.

More work is required to test for effects of selenium compounds under different conditions. Daphnia exhibited a threshold effect at 2.5 mg l of selenium in a 48-hour exposure at 23 C (Bringmann and Kuhn 1959a).[231] Barnhart (1958)[118] reported that mortalities of fish stocked in a Colorado reservoir were caused by selenium leached from bottom deposits, passed through the food chain, and accumulated to lethal concentrations by the fish in their liver.

Recommendation

In view of the possibility that selenium may be passed through the food chain and accumulated in fish, it is recommended that an application factor of 0.01 be applied to marine 96-hour LC50 data for the appropriate organisms most sensitive to selenium. On the basis of data available at this time, it is suggested that concentrations of selenium equal to or exceeding 0.01 mg/l constitute a hazard in the marine environment, and levels less than 0.005 mg/l present minimal risk of deleterious effects.

Silver

Silver is one of the more commercially important metals; 4,938 tons were consumed in the U.S. during 1968, excluding that used for monetary purposes (U.S. Department of the Interior, Bureau of Mines 1969).[289] It is the best known conductor of heat and electricity. Although not oxidized by air, silver is readily affected by hydrogen sulfide to form the black silver sulfide.

Silver has many uses. In addition to making currency, it is used for photographic purposes, for various chemical purposes, and also in jewelry making and in silverplating of cutlery.

Silver is toxic to aquatic animals. Concentrations of 400 μg/l killed 90 per cent of test barnacles (*Balanus balanoides*) in 48 hours (Clarke 1947).[143] Concentrations of silver nitrate from 10 to 100 μg/l caused abnormal or inhibited development of eggs of *Paracentrotus* and concentrations of 2 μg/l of silver nitrate delayed development and caused deformation of the resulting plutei (Soyer 1963).[266] Adverse effects occurred at concentrations below 0.25 μg/l of silver nitrate, and several days were required to eliminate adverse effects by placing organisms in clean water (Soyer 1963).[266] Silver nitrate effects on development of *Arbacia* have been reported at approximately 0.5 μg/l (Soyer 1963,[266] Wilber 1969[299]). In combination with silver, copper acts additively on the development of *Paracentrotus* eggs (Soyer 1963).[266] On a comparative basis on studies on Echinoderm eggs (Soyer 1963),[266] silver has been found to be about 80 times as toxic as zinc, 20 times as toxic as copper, and 10 times as toxic as mercury.

Calabrese et al. (*unpublished manuscript*)[310] noted an LC50 of 0.006 mg/l silver for eggs of the American oyster (*Crassostrea virginica*). Jones (1948)[200] reported that the lethal concentration limit of silver, applied as silver nitrate, for sticklebacks (*Gasterosteus aculeatus*) at 15 to 18 C was 0.003 mg l, which was confirmed approximately by Anderson (1948),[112] who found 0.0048 mg/l to be the toxic threshold for sticklebacks. Jackim et al. (1970)[196] reported adverse effects on the liver enzymes of the killifish *Fundulus heteroclitus* at 0.04 mg l of silver.

The sublethal responses to silver compounds may be great, in view of the effects on developing eggs; and further research should be conducted on effects of sublethal concentrations of silver compounds by themselves and in combination with other chemicals. The disruption of normal embryology or of nutrition could be of much greater importance than direct mortality in the perpetuation of the species.

Concentrations of silver cannot exceed that permitted by the low solubility product of silver chloride. However, silver complexes may be present, and their effects are unknown.

Recommendation

It is recommended that the concentrations of silver in marine waters not exceed 0.05 of the 96-hour LC50 for the appropriate species most sensitive to silver. On the basis of data available at this time, it is suggested that concentrations of silver equal to or exceeding 5 μg/l constitute a hazard to the marine environment, and levels less than 1 μg/l present minimal risk of deleterious effects.

Sulfides

Sulfides in the form of hydrogen sulfide have the odor of rotten eggs and are quite toxic. Hydrogen sulfide is soluble in water to the extent of 4000 mg/l at 20 C and 1 atmosphere. Sulfides are produced as a by-product in tanneries, chemical plants, and petroleum refineries, and are used in pulp mills, chemical precipitation, and in chemical production. Hydrogen sulfide is produced in natural decomposition processes and in anaerobic digestion of sewage and industrial wastes. Sulfate in sea water is reduced to sulfide in the absence of oxygen. In the presence of certain sulfur-utilizing bacteria, sulfides can be oxidized to colloidal sulfur. At the normal pH and oxidation-reduction potential of aerated sea water, sulfides quickly oxidize to sulfates.

Hydrogen sulfide dissociates into its constituent ions in two equilibrium stages, which are dependent on pH (McKee and Wolf 1963).[226]

The toxicity of sulfides to fish increases as the pH is lowered because of the HS^- or H_2S molecule (Southgate 1948).[265] Inorganic sulfides are fatal to sensitive species such as trout at concentrations of 0.05 to 1.0 mg l, even in neutral and somewhat alkaline solutions (Doudoroff 1957).[149] Hydrogen sulfide generated from bottom deposits was reported to be lethal to oysters (de Oliveira 1924).[145]

Bioassays with species of Pacific salmon (*Oncorhynchus* *tshawytscha, O. kisutch*) and sea-run trout (*Salmo clarkii clarkii*) showed toxicity of hydrogen sulfide at 1.0 mg/l and survival without injury at 0.3 mg/l (Van Horn et al. 1949,[291] Dimick 1952,[147] Haydu et al. 1952,[179] Murdock 1953,[234] Van Horn 1959[290]). Holland et al. (1960)[180] reported that 1 mg/l of sulfide caused loss of equilibrium in 2 hours, first kills in 3 hours, and 100 per cent mortality in 72 hours with Pacific salmon.

Hydrogen sulfide in bottom sediments can affect the maintenance of benthic invertebrate populations (Thiede et al. 1969).[280] The eggs and juvenile stages of most aquatic organisms appear to be more sensitive to sulfides than do the adults. Adelman and Smith (1970)[107] noted that hydrogen sulfide concentrations of 0.063 and 0.020 mg/l killed northern pike (*Esox lucius*) eggs and fry, respectively; and at 0.018 and 0.006 mg/l, respectively, reduced survival, increased anatomical malformations, or decreased length were reported.

Recommendation

It is recommended that an application factor of 0.1 be applied to marine 96-hour LC50 for the appropriate organisms most sensitive to sulfide. On the basis of data available at this time, it is suggested that concentrations of sulfide equal to or exceeding 0.01 mg/l constitute a hazard in the marine environment, and levels less than 0.005 mg/l present minimal risk of deleterious effects, with the pH maintained within a range of 6.5 to 8.5.

Thallium

Thallium salts are used as poison for rats and other rodents and are cumulative poisons. They are also used for dyes, pigments in fireworks, optical glass, and as a depilatory.

Thallium forms alloys with other metals and readily amalgamates with mercury. It is used in a wide variety of compounds. Nehring (1963)[238] reported that thallium ions were toxic to fishes and aquatic invertebrates. The response of fishes to thallium poisoning is similar to that of man, an elevation in blood pressure. In both the fish and invertebrates, thallium appears to act as a neuro-poison (Wilber 1969).[299]

Adverse effects of thallium nitrate have been reported for rainbow trout (*Salmo gairdneri*) at levels of 10 to 15 mg/l; for perch (*Perca fluviatilis*) at levels of 60 mg/l; for roach (*Rutilus rutilis*) at levels of 40 to 60 mg/l; for water flea (*Daphnia* sp.) at levels of 2 to 4 mg/l; and for *Gammarus* sp. at levels of 4 mg/l. The damage was shown within three days for the various aquatic organisms tested. Damage also resulted if the fish were exposed to much lower concentrations for longer periods of time (Wilber 1969).[299]

Recommendation

Because of a chronic effect of long-term exposure of fish to thallium, tests should be conducted for at least 20 days on sensitive species. Techniques should measure circulatory disturbances (blood pressure) and other sublethal effects in order to determine harmful concentrations. The concentration in sea water should not exceed 0.05 of this concentration. On the basis of data available at this time, it is suggested that concentrations of thallium equal to or exceeding 0.1 mg/l constitute a hazard in the marine environment, and levels less than 0.05 mg/l present minimal risk of deleterious effects.

Uranium

Uranium is present in wastes from uranium mines and nuclear fuel processing plants, and the uranyl ion may naturally occur in drainage waters from uranium-bearing ore deposits. Small amounts may also arise from its use in tracer work, chemical processes, photography, painting and glazing porcelain, coloring glass, and in the hard steel of high tensile strength used for gun barrels.

Many of the salts of uranium are soluble in water, and it is present at about 3 μg/l in sea water. A significant proportion of the uranium in sea water is in the form of stable complexes with anionic constituents. It has been estimated that uranium has a residence time of 3×10^5 years in the oceans (Goldberg et al. 1971),[illegible] a span that makes it one of the elements with the slowest turnover time. Uranium is stabilized by hydrolysis which tends to protect it against chemical and physical interaction and thus prevents its removal from sea water. The salts are considered to be 4 times as germicidal as phenol to aquatic organisms.

Natural uranium (U-238) is concentrated from water by the algae *Ochromonas* by a factor of 330 in 48 hours (Morgan 1961).[232] Using River Havel water, Bringmann and Kuhn (1959a,[131] 1959b[132]) determined the threshold effect of uranyl nitrate, expressed as uranium, at 28 mg/l on a protozoan (*Microregma*), 1.7 to 2.2 mg/l on *Escherichia Coli*, 22 mg/l on the alga *Scenedesmus*, and 13 mg/l on *Daphnia*. Tarzwell and Henderson (1956)[277] found the sulfate, nitrate, and acetate salts of uranium considerably more toxic to fathead minnows (*Pimephales promelas*) on 96-hour exposure in soft water than in hard water, the 96-hr LC50 for uranyl sulfate being 2.8 mg/l in soft water and 135 mg/l in hard water.

The sparse data for uranium toxicity in sea water suggest that uranyl salts are less toxic to marine organisms than to freshwater organisms. Yeasts in the Black Sea were found to be more active than the bacteria in taking up uranium (Pshenin 1960).[261] Studies by Koenuma (1956)[illegible] showed that the formation of the fertilization membrane of *Urechis* eggs was inhibited by 250 mg/l of uranyl nitrate in sea water, and that this concentration led to polyspermy.

Recommendation

It is recommended that an application factor of 0.01 be applied to marine 96-hour LC50 data for

the appropriate organisms most sensitive to uranium. On the basis of data available at this time it is suggested that concentrations of uranium equal to or exceeding 0.5 mg/l constitute a hazard in the marine environment, and levels less than 0.1 mg/l present minimal risk of deleterious effects.

Vanadium

Vanadium occurs in various minerals, such as chileite and vanadinite. It is used in the manufacture of vanadium steel. Vanadates were used at one time to a small extent for medicinal purposes. Vanadium has been concentrated by certain marine organisms during the formation of oil-bearing strata in geological time. Consequently, vanadium enters the atmosphere through the combustion of fossil fuels, particularly oil. In addition, eighteen compounds of vanadium are used widely in commercial processes (Council on Environmental Quality 1971).[144]

Recommendation

It is recommended that the concentration of vanadium in sea water not exceed 0.05 of the 96-hour LC50 for the most sensitive species.

Zinc

Most of the available information on zinc toxicity is for freshwater organisms, and for this reason the reader is referred to the discussion of zinc in Section III, p. 182.

Recommendation

Because of the bioaccumulation of zinc through the food web, with high concentrations occurring particularly in the invertebrates, it is recommended that an application factor of 0.01 be applied to marine 96-hour LC50 data for the appropriate organisms most sensitive to zinc. On the basis of data available at this time, it is suggested that concentrations of zinc equal to or exceeding 0.1 mg/l constitute a hazard in the marine environment, and levels less than 0.02 mg/l present minimal risk of deleterious effects.

It should be noted that there is a synergistic effect when zinc is present with other heavy metals, e.g., Cu and Cd, in which case the application factor may have to be lowered by an order of magnitude (LaRoche 1972).[211]

OIL IN THE MARINE ENVIRONMENT

Oil is becoming one of the most widespread contaminants of the ocean. Blumer (1969)[219] has estimated that between 1 and 10 million metric tons of oil may be entering the oceans from all sources. Most of this influx takes place in coastal regions, but oil slicks and tar balls have also been observed on the high seas (Horn et al. 1970,[224] Morris 1971[243]). Collections of tar balls were made by towing a neuston net which skims the surface, and the investigators found that the tar balls were more abundant than the normal sargassum weed in the open Atlantic, and that their nets quickly became so coated with tar and oil that they were unusable. Thus, oil pollution of the sea has become a global problem of great, even though as yet inadequately assessed, significance to the fisheries of the world.

Sources of Oil Pollution

Although accidental oil spills are spectacular events and attract the most public attention, they constitute only about 10 per cent of the total amount of oil entering the marine environment. The other 90 per cent originates from the normal operation of oil-carrying tankers, other ships, off-shore production, refinery operations, and the disposal of oil-waste materials (Table IV–4).

Two sources of oil contamination of the sea not listed in Table IV–4 are the seepage of oil from underwater oil reservoirs through natural causes and the transport of oil in the atmosphere from which it precipitates to the surface of the sea. Natural seepage is probably small compared to the direct input to the ocean (Blumer 1972);[220] but the atmospheric transport, which includes hydrocarbons that have evaporated or been emitted by engines after incomplete combustion, may be greater than the direct input.

Some of these sources of oil pollution can be controlled more rigorously than others, but without application of adequate controls wherever possible the amount of petroleum hydrocarbons entering the sea will increase. Our technology is based upon an expanding use of petroleum; and the production of oil from submarine reservoirs and the use of the sea to transport oil will both increase. It is estimated that the world production of crude oil in 1969 was nearly 2 billion tons; on this basis total losses to the sea are somewhat over 0.1 per cent of world production.

TABLE IV–4—Estimated Direct Petroleum Hydrocarbon Losses to the Marine Environment (Airborne Hydrocarbons Deposited on the Sea Surface are Not Included)
(Millions of tons)

	1969	1975 (estimate)ᵃ		1980 (estimate)	
		Min	Max	Min	Max
1. Tankers	.530	.155	.825	.075	1.062
2. Other ships	.500	.765	.785	.940	.940
3. Offshore production	.100	.160	.320	.230	.460
4. Refinery operations	.300	.220	.450	.440	.650
5. Oil wastes	.550	.825	.925	1.200	1.200
6. Accidental spills	.200	.300	.300	.440	.440
TOTAL	2.180	2.245	3.605	3.325	4.752
Total Crude Oil Production	1820	2700		4000	

ᵃ The minimum estimates assume full use of known technology; the maximums assume continuation of present practices.

Revelle et al. 1972[344].

Some losses in the exploitation, transportation, and use of a natural resource are inevitable; but if this loss ratio cannot be radically improved, the oil pollution of the ocean will increase as our utilization increases.

Biological Effects of Petroleum Hydrocarbons

Description of Oil Pollution Oil is a mixture of many compounds, and there are conflicting views concerning its toxicity to marine organisms. Crude oils may contain thousands of compounds, and will differ markedly in their composition and in such physical properties as specific gravity, viscosity, and boiling-point distribution. The hydrocarbons in oil cover a wide range of molecular weights from 16 (methane) to over 20,000. Structurally, they include aliphatic compounds with straight and branched chains, olefins, and the aromatic ring compounds. Crude oils differ mainly in the relative concentrations of the individual members of these series of compounds. The various refinery processes to which oil is subjected are designed to isolate specific parts of the broad spectrum of crude oil compounds, but the refined products themselves remain complex mixtures of many types of hydrocarbons.

In spite of the many differences among them, crude oils and their refined products all contain compounds that are toxic to species of marine organisms. When released to the marine environment, these compounds react differently. Some are soluble in the water; others evaporate from the sea surface, form extensive oil slicks, or settle to the bottom if sand becomes incorporated in the oil globule. More complete understanding of toxicity and the ecological effects of oil spills will require studies of the effects of individual components, or at least of classes of components, of the complex mixture that made up the original oil. The recent development of gas chromatography has made it possible to isolate and identify various fractions of oil and to follow their entry into the marine system and their transfer from organism to organism.

An oil slick on the sea surface can be visually detected by iridescence or color, the first trace of which is formed when 100 gallons of oil spread over 1 square mile (146 liters km^2) (American Petroleum Institute 1949).[317] The average thickness of such a film is 0.145 microns. Under ideal laboratory conditions, a film 0.038 microns thick can be detected visually (American Petroleum Institute 1963).[318] For remote sensing purposes, oil films with a thickness of 100 microns can be detected using dual polarized radiometers, 1 micron using radar imagery, and 0.1 microns using multispectral imagery in the UV region (Catoe and Orthlieb 1971).[323] A summary of remote sensing capabilities is presented in Table IV-5. Because remote sensing is less effective than the eye in detecting surface oil, any concentration of oil detectable by remote means currently available will exceed the recommendations given below.

The death of marine birds from oiling is one of the earliest and most obvious effects of oil slicks on the sea surface. Thousands of seabirds of all varieties are often involved in a large spill. Even when the birds are cleaned, they frequently die because the toxic oil is ingested in preening their feathers. Dead oiled birds are often found along the coast when no known major oil spill has occurred, and the cause of death remains unknown.

When an oil spill occurs near shore or an oil slick is brought to the intertidal zone and beaches, extensive mortality of marine organisms occurs. When the Tampico Maru ran aground off Baja California in 1957, about 60,000 barrels of spilled diesel fuel caused widespread death among lobsters, abalones, sea urchins, starfish, mussels, clams, and hosts of smaller forms (North 1967).[344] A beneficial side effect of this accident was also noted by North. When the sea urchins that grazed on the economically important kelp beds of the area were killed in massive numbers by the oil spill, huge canopies of kelp returned within a few months (see p. 237). The oil spills from the wreck of the tanker Torrey Canyon and the Santa Barbara oil well blowout both involved crude oil, and in both cases oil reached the beaches in variable amounts some time after release. The oil may thus have been diluted and modified by evaporation or sinking before it reached the beach. In the Santa Barbara spill many birds died, and entire plant and animal communities in the intertidal zone were killed by a layer of encrusting oil often 1 or 2 centimeters thick (Holmes 1967).[337] At locations where the oil film was not so obvious, intertidal organisms were not severely damaged (Foster et al. 1970).[327] In the case of the Torrey Canyon, the deleterious effects have been attributed more to the detergents and dispersants used to control the oil than to the oil itself (Smith 1968).[347]

A relatively small oil spill in West Falmouth, Massachusetts, occurred within a few miles of the Woods Hole Oceanographic Institution in September 1969. An oil barge, the Florida, was driven onto the Buzzards Bay Shore where it released between 650 and 700 tons of No. 2 fuel oil into the coastal waters. Studies of the biological and chemical effects of this spill are continuing, more than two years after the event (Blumer 1969,[319] Hampson and Sanders 1969,[331] Blumer et al. 1970,[322] Blumer and Sass 1972[321]). Massive destruction of a wide range of fish, shellfish, worms, crabs, other crustaceans, and invertebrates occurred in the region immediately after the accident. Bottom-living fish and lobsters were killed and washed ashore. Dredge samples taken in 10 feet of water soon after the spill showed that 95 per cent of the animals recovered were dead and the others moribund. Much of the evidence of this immediate toxicity disappeared within a few days, either because of the breaking up of the soft parts of the organism, burial in the sediments, or dispersal by water currents. Careful chemical and biological analyses reveal, however, that not only has the damaged area been slow to recover but the extent of the damage has been expanding with time. A year and a half after the spill, identifiable

TABLE IV-5—Summary of Remote Sensor Characteristics For Oil Detection

Wave length	Detection mechanism	Performance summary	Possible sensor configuration					Comments
			Type	Resolution	Weight	Volume	Swath width	
Ultraviolet (≤0.4 μm).	Reflectance differential (Oil/Water contrast) Fluorescence	Reflective signature a. Repeatable positive response from thin slicks (~.1 micron). b. Variable response from thicker slicks dependent upon oil type, water quality and illumination conditions. c. Atmospheric haze limitations major. d. Signal limitations prevent night-time detection.	UV Vidicon	800 lines/frame (high scene illumination) 100-200 lines/frame (low scene illumination)	33 lbs.	2 cu. ft.	40° FOV (727 ft @ 10 K)	Developed component available for UV vidicon and/or scanner. Integration ver. with CRT display. Line scanner may require data buffer for high resolution, real time display, or film processor
		Fluorescence signature	UV Scanner	2 mr	80 lbs.	3.6 cu. ft.	2.7 mi @ 10 K	
		1. Artificial Excitation (narrow-band) a. Spectral character strongly correlated to oil thickness. b. Intensity strongly correlated to oil type API) and oil thickness, weakly correlated to temperature. c. Decay characteristics moderately to strongly correlated to oil type, uncorrelated to oil thickness. d. All characteristics independent of ambient illumination conditions.	Pulsed Laser	1 mr	180 lbs.	4 cu. ft.	10 ft @ 10 K	Effective against thin and thick slicks under solar or artificial illumination. Active laser system sensitivity limitations hinder use in detection or mapping mode. Identification capability very good with moderate to good thickness determination.
		2. Solar excitation (broad-band) a. Spectral character moderately to weakly correlated to oil type and thickness. b. Intensity strongly correlated to oil type, oil thickness and ambient illumination conditions. c. Decay characteristics not detectable. d. Signal limitations prevent operation except under strong solar illumination.						
Visible (0.4 to .7 μm)..	Reflectance Differential (Oil/Water Contrast)	Reflective Signature	Aerial Cameras					
		a. Variable response from all slicks dependent upon thickness, oil type, water quality and illumination conditions	KC-8	2 ft @ 10 K	190 lbs.	11.70 cu. ft.	74° FOV 3.5 mi. @ 10 K	Aerial cameras real time display not possible.
		b. Signal limitations prevent moonless nighttime detection.	[illegible]	3.0 ft @ 10 K	16 lbs.	.4 cu. ft.		Sensitivity limitations prevent night-time operations.
		c. False alarm problem significant. d. Atmospheric haze limitation major.	KA-62	" "	81.5 lbs.	5.24 cu. ft.		Compensation for atmospheric haze difficult.
		e. Maximum contrast between oil and water occurs at (.30 to .45 μm) and (.6 to .80 μm). f. Minimum contrast between oil and water occurs at (.45 to .60 μm) g. Best contrast achieved with overcast sky.	Vidicon	800 lines/frame	33 lbs.	2 cu. ft.	40° FOV with zoom lens 7270 ft. at 10 K	UV photography great potential for detecting oil. Color is good; however, sunlight gives false responses. Panchromatic, IR, and color photography and TV give good results only when oil is thick and ropy. Vidicons useful for real-time detection and mapping at various wave lengths, giving option for good detection with negligible false alarms for day operation and fair-to-good detection with low false alarms for night operation. Display characteristics optimum for surveillance.
Infrared		Reflective Signature						
Near Infrared (0.8 to 0.1 μm)	Reflectance Differential (Oil/Water Contrast)	a. Repeatable positive response from all slicks under all conditions.	Line Scanner	2 mr	80 lbs.	4.0 cu. ft.	2.7 mi @ 10 K	Line scanner oil-slick response variable but essentially predictable, but may have some false alarm problems.
Far Infrared (8 to 14 μm)	Thermal Emission Differential	b. Moonless night-time detection capability. c. False alarm problems negligible. d. Atmospheric haze limitation moderate.	Framing Scanner	4 mr	220 lbs	3.5 cu. ft.	23° FOV	

TABLE IV-5—Summary of Remote Sensor Characteristics For Oil Detection—Continued

Wavelength	Detection mechanism	Performance summary	Possible sensor configuration					Comments
			Type	Resolution	Weight	Volume	Swath width	
		Thermal Signature a. Variable response dependent greatly upon oil type and dependent significantly upon thickness and solar heating. Variability predictable to significant degree (slicks > 10 μm) b. Day night detection independent of illumination conditions. c. False alarm problem slight. d. Atmospheric haze limitations moderate to slight.						Day night detection under VFR conditions. Real time display capabilities good but limited to "single-look" display generation. Developed equipment available.
Microwave	Emissive Differential (Oil Water Contrast) Wave Structure Modification	Emissive Signature a. Emissivity of petroleum products is significantly higher than that of a calm sea surface. b. Crude oil pollutants have decreasing dielectric constants (increasing emissivity) with increasing API gravity. c. Microwave signature of oil film inversely proportional to sensor wave length. d. The horizontal polarized microwave signature of oil is twice the vertically polarized signature of an oil slick on a flat water surface. e. Detection improves with decreasing sensor wave lengths and becomes poorer as the sea state increases. f. Atmospheric cloud limitations moderate to slight. g. Can effectively detect slicks less than 0.1 mm at viewing. h. Dual frequency microwave techniques show great promise in measuring oil slick thickness.	Line Scanning Imager	1.0°	60 lbs.	3 cu. ft.	2.7 mi @ 10 K	Clouds that are raining between sensor and slick as well as very high sea states hamper performance. Technology for equipment development available. Real time display consists of facsimile and/or CRT.
Radar	Wave Structure Modification Scattering Cross-section Differential	Reflective Signature a. Oil film on surface of water suppresses capillary which results in a significant difference in energy back scattered from contaminated surface and that scattered from surrounding clean water (from oil slicks very little energy back scattered by three orders of magnitude). b. Vertical polarization capable of detecting and mapping oil slick less than 1 micron. c. Atmospheric cloud limitations slight.	Forward Scanning (35 GHz)	100×100 ft²	~800 lbs.	10 cu. ft.	30 mi @ 12 K	Technology exists for equipment development of forward scanning and synthetic aperature radar.
			Synthetic Aperture (3.3 GHz)	100×100 ft²	~1200 lbs.	17 cu. ft.	100 mi @ 36 K	Real time display possible for forward scanning radar via facsimile and/or CRT; synthetic aperature radar requires optical processing.

fractions of the source oil were found in organisms that still survived on the perimeter of the area. Hydrocarbons ingested by marine organisms may pass through the wall of the gut and become part of the lipid pool (Blumer et al. 1970).[illegible] When dissolved within the fatty tissues of the organisms, even relatively unstable hydrocarbons are preserved. They are protected from bacterial attack and can be transferred from food organism to predators and possibly to man.

The catastrophic ecological effects of the oil spills of the Tampico Maru, and the Florida appear to be more severe than those reported from other oil spills such as the Torrey Canyon and the Santa Barbara blowout. The Tampico Maru and the Florida accidents both released refined oils (in one case diesel oil and in the other, No. 2 fuel oil) and both occurred closer to shore than either the Torrey Canyon or the Santa Barbara accidents which released crude oil. The differences in the character of the oil and the proximity to shore may account for the more dramatic effects of the first two accidents, but it is clear that any release of oil in the marine environment carries a threat of destruction and constitutes a danger to world fisheries.

Persistence of Oil in the Ocean As mentioned above, oil can be ingested by marine organisms and incorporated in their lipid pool. Hydrocarbons in the sea are also degraded by marine microorganisms. Very little is known as yet about the rate of this degradation, but it is known that no single microbial species will degrade any whole crude oil. Bacteria are highly specific, and several species will probably be necessary to decompose the numerous types of hydrocarbons in a crude oil. In the process of decomposition, intermediate products will be formed and different species of bacteria and other microorganisms may be required to attack these decomposition products (ZoBell 1969).[349]

The oxygen requirement of microbial oil decomposition is severe. The complete oxidation of one gallon of crude oil requires all the dissolved oxygen in 320,000 gallons of air-saturated sea water (ZoBell 1969).[349] It is clear that oxidation might be slow in an area where previous pollution has depleted the oxygen content. Even when decomposition of oil proceeds rapidly, the depletion of the oxygen content of the water by the microorganisms degrading the oil may have secondary deleterious ecological effects. Unfortunately, the most readily attacked fraction of crude oil is the least toxic, i.e., the normal paraffins. The more toxic aromatic hydrocarbons, especially, the carcinogenic polynuclear aromatics, are not rapidly degraded.

That our coastal waters are not devoid of marine life, after decades of contamination with oil, indicates that the sea is capable of recovery from this pollution. However, increasing stress is being placed on the estuarine and coastal environment because of more frequent oil pollution incidents near shore; and once the recovery capacity of an environment is exceeded, deterioration may be rapid and catastrophic. It is not known how much oil pollution the ocean can accept and recover from, or whether the present rate of addition approaches the limit of the natural system.

It appears that the oceans have recovered from the oil spilled during the six years of the second World War, though some unexplained recent oil slicks have been attributed to the slow corrosion of ships sunk during that conflict. It has been estimated (SCEP)[343] that during the war, the United States lost 98 vessels with a total oil capacity of about 1 million tons, and that another 3 million tons of oil were lost through the sinking of ships of other combatants during the same period. These losses were large in the context of the 1940's, but the total for that period was only about twice the annual direct influx to the ocean at the present time. Although no extensive deleterious effects of these sinkings and oil releases on the fisheries catch of the world have been found, it must be emphasized again that when a pollutant is increasing yearly in magnitude past history is not a reliable source of prediction of future effects.

The Toxicity of Oil There is a dearth of dependable observations on the toxicity of oil to marine organisms. It is difficult to evaluate the toxicity of this complex mixture of compounds which is not miscible with sea water. A variety of techniques have been used which are not intercomparable. In some experiments, oil is floated on the water in the test container, and the concentration given is derived from the total quantity of oil and the total quantity of water. This is clearly not the concentration to which the organism has been exposed. In other experiments, extracts of oil with hot water or with various solvents have been added to the test jar without identification of the oil fraction being tested. In still other cases, care has been taken to produce a fine emulsion of oil in sea water more representative of the actual concentration to which the test organism is exposed. Considering the differences in the meaning of "concentration" in these tests and the variation in sensitivity of the test organisms, it is not surprising that the ranges of toxicity that can be found in the literature vary by several orders of magnitude.

Studies of the biological effects of oil have been reviewed by Clark (1971).[225] Mironov (1971)[342] carried out toxicity studies by comparable techniques using a variety of marine organisms. In testing eleven species of phytoplankton he found that cell division was delayed or inhibited by concentrations of crude oil (unspecified type) ranging from [illegible] to 1000 ppm. He also showed that some copepods were sensitive to a 1 ppm suspension of fresh or weathered crude oil and of diesel oil. Freegarde et al. (1970)[226] found that the larvae of *Ballanus ballanoides* and adult *Calanus* copepods maintained in a suspension of crude oil ingest, without apparent harm, droplets of oil that later appear in the feces. Mironov (1967)[311] found 100 per cent mortality of developing flounder spawn at concentrations of three types of oil ranging from 1 to 100 ppm and an increased abnormality of development at longer periods of time in concentrations as low as 0.01 ppm. In contrast other experimenters have found that concentrations of several per cent are necessary to kill adult fish in a period of a few days (Chipman and Galtsoff 1949,[224] Griffith 1970[229]).

The evidence is clearer that a combination of oil and detergents is more toxic than oil alone. This was first definitely established in studies of the Torrey Canyon spill (Smith 1968),[347] and the toxicity of the various detergents used in this operation is discussed by Corner et al. (1968).[illegible] The four detergents tested were all more toxic than Kuwait crude oil, and all showed signs of toxicity between 2 and 10 ppm. The solvents used with these detergents were also highly toxic but tended to lose their toxicity over time through evaporation. A bioassay test carried out by the Michigan Department of Natural Resources (1969)[illegible] revealed that the least toxic detergent mixed with oil could be a hundred times as concentrated (1800 ppm) as the most toxic (14 ppm) and cause the same toxic effect. La Roche et al. (1970)[337] defined bioassay procedures for oil and oil dispersant toxicity evaluation using fish, *Fundulus heteroclitus*, and the sandworm, *Nereis virens* (Table IV-[illegible]).

The mortality of seabirds as a result of oil pollution is direct and immediate, and in a major oil spill, is measured in the thousands. The diving birds which spend most of their life at sea are most prone to death from oil pollution, but any bird that feeds from the sea or settles on it is vulnerable. In oil-matted plumage air is replaced by water

TABLE IV-6—Determinations (Summarized) of Acute Toxicities of 10 Chemical Dispersants Alone and in Combination with Crude Oil to Sandworm (Nereis virens) *and Mummichog* (Fundulus heteroclitus) *in Laboratory Bioassay Tests*

Substance	96 hour LC50 (ml/l)	
	Nereis	Fundulus
Crude oil A		16.5
Crude oil B	6.1	8.2
Oil and dispersants[a]	.055-.761	.187-1
Dispersants	.007-7.10	.006-2

[a] Ranges of values for 10 dispersants mixed 1 part dispersant to 10 parts of oil by volume.
LaRoche et al. 1970[337].

causing loss of both insulation and buoyancy, and oil ingested during preening can have toxic effects.

Hartung and Hunt (1966)[332] fed oils directly to birds by stomach tube and later analyzed the pathological and physiological effects through autopsies. The lethal dose for three types of oil ranged from 1 ml to 4 ml per kilogram (ml/kg) when the birds were kept outdoors under environmental stress. The experimenters concluded that a duck could typically acquire a coating of 7 grams of oil and would be expected to preen approximately 50 per cent of the polluting oil from its feathers within the first few days. Enough of this could easily be ingested to meet the lethal dosage of 1 to 4 ml/kg. Thus, birds that do not die promptly from exposure to cold or by drowning as a result of oil pollution may succumb later from the effects of ingestion.

Corrective Measures

The only effective measure for control of oil pollution in the marine environment is prevention of all spills and releases. The time-lag involved in corrective methods means that some damage will inevitably occur before the corrective measures take effect. Furthermore, the soluble parts of the oil already in the water will not be removed by any of the present methods of post-spill cleanup.

Control measures have been introduced that appreciably reduce excessive oil pollution from normal tanker operations (see Table IV-4). The load on top (LOT) process concentrates waste oil that is ultimately discharged with the new cargo (IMCO 1965a,[335] 1965b[336]). This procedure recovers somewhat more than 98 per cent of oil that would otherwise be released to the sea. It has been estimated (Revelle et al. 1972)[345] that 80 per cent of the world fleet uses these control measures today, and if they continue to do so faithfully these ships will contribute only 3.0×10^4 tons of the total tonnage of oil loss. In contrast, the 20 per cent of the fleet not using these control measures contributes 5×10^6 tons. If these control measures were not in use by a major fraction of the tanker fleet, the contamination of the sea from this source would be about five times greater than it is today.

Among the earliest methods for the cleanup of spilled oil was to pick up or bury the material that came ashore while disregarding the oil that remained at sea. It was found that the use of straw to absorb the oil made this cleanup procedure easier, and in the cleanup of the Arrow oil spill (Ministry of Transport, Canada 1970).[illegible] peat moss was found to be an effective absorbent for Bunker C oil. Recent studies promise mechanical means for handling and cleaning sand contaminated with oil by use of earth moving equipment. fluid-bed, and froth flotation techniques (Guntz and Meloy 1971,[330] Mikolaj and Curran 1971.[illegible] Sartor and Foget 1971).[346]

The use of detergents to treat oil slicks is essentially cosmetic. It removes the obvious evidence of oil and for that reason appeals to the polluter. However, after treatment with detergent, the oil is dispersed in the form of fine droplets and becomes even more available to the biota of the sea than it would be if it were left in the form of a surface film. Because of the finer degree of dispersion, the soluble toxic fractions dissolve more rapidly and reach higher concentrations in sea water than would result from natural dispersal. The droplets themselves may be ingested by filter-feeding organisms and thus become an integral part of the marine food chain. Some of the oil may pass through the gut in the feces of these organisms, but Blumer et al. (1970)[322] have shown that it can pass through the gut wall and be incorporated in the organism's lipid pool. It can thus be transferred from organism to organism and, potentially, into the food that man takes from the ocean for his use.

Sinking of oil has been achieved by scattering talc or chalk on the oil causing it to agglutinate into globules of greater density than sea water. Such sunken oil tends to kill bottom fauna before even the motile bottom dwellers have time to move away. The sessile forms of commercial importance, such as clams, oysters and scallops, cannot escape, and other motile organisms such as lobsters (*Homarus americanus*) may actually be attracted in the direction of the spill where exposure will contaminate or kill them. Little is known about the rate of degradation of oil in bottom sediments, but it is known that some fractions will persist for over two years (Blumer 1969.[illegible] Blumer and Sass 1972[321]). Chipman and Galtsoff (1949)[illegible] showed that the toxicity of oil is not diminished by adsorption on carbonized sand which can be used as a sinking agent.

Efforts were made to burn the oil in both the Torrey Canyon and the Wafra, which was wrecked off the coast of South Africa in 1971. When oxidation is complete, oil is converted to carbon dioxide and water and removed as a pollutant. Burning oil within a tanker, however, is difficult; and it has not been successful even when oxidants are added. Volatile fractions may burn off quickly, but most of the oil resists combustion. Incomplete combustion is therefore not only more common, but the smoke and volatile oils themselves become atmospheric pollutants many of which ultimately return to the sea through precipitation and accumulation on the water surface. Oil can be burned on the surface of the sea by using wicks or small glass beads to which the oil clings thus removing itself from the quenching effects of the water. The use of "seabeads" was successful in burning Bunker C oil on the beach and moderately successful in burning a slick in two to three foot seas in the cleanup following the wreck of the Arrow (Ministry of Transport, Canada 1970).[440] However, during burning, the elevated temperature of the oil increases the solubility in water of the most toxic components, and this can cause greater biological damage than if the oil is left unburned.

Mechanical containment and removal of oil appear to be ideal from the point of view of avoiding long-term biological damage, but however promptly such measures are taken, some of the soluble components of the oil will enter the water and it will not be possible to remove them. A variety of mechanisms for containing oil have been proposed, such as booms with skirts extending into the water. Various surface skimmers to collect oil and pump it into a standby tanker have been conceived. Unfortunately, most wrecks occur during less than ideal weather conditions which makes delivery and deployment of mechanical devices difficult. Floating booms are ineffective in a rough sea because even if they remain properly deployed, oil can be carried over the top of them by wind and splashing waves or under them by currents. In protected waters, however, recovery can be quite effective, and among the methods of oil removal used today, booms are one of the most effective if conditions for their use are favorable.

Microbiological degradation is the ultimate fate of all oil left in the sea, but as was mentioned previously, the oxygen requirement for this is severe. There is also the problem of providing other nutrients, such as nitrogen and phosphorus, for the degrading bacteria. Nevertheless, this process is a "natural" one, and research into increasing the rate of bacteriological degradation without undesirable side effects is to be encouraged.

Although an ultimate solution to the cleanup of oil spills is desperately needed, prevention of spills remains the most effective measure. When wrecks occur, every effort should be made to offload the oil before it enters the marine environment. Oil spills that occur in harbors during transfer of oil to a refinery or of refined oil to a tanker should be more easily controlled. Portable booms could confine any oil released and make possible recovery of most harbor spillage. Available technology is adequate to prevent most accidental spills from offshore well drilling or operations. It is necessary to require that such technology be faithfully employed.

Recommendations

No oil or petroleum products should be discharged into estuarine or coastal waters that:

- **can be detected as a visible film, sheen, or discoloration of the surface, or by odor;**
- **can cause tainting of fish or edible invertebrates or damage to the biota;**
- **can form an oil deposit on the shores or bottom of the receiving body of water.**

In this context, discharge of oil is meant to include accidental releases that could have been prevented by technically feasible controls.

Accidental releases of oil to the marine environment should be reclaimed or treated as expeditiously as possible using procedures at least equivalent to those provided in The National Contingency Plan of 1970. The following recommendations should be followed to minimize damage to the marine biota.

- **Oil on the sea surface should be contained by booms and recovered by the use of surface skimmers or similar techniques.**
- **In the event of a tanker wreck, the oil remaining in the hulk should be off-loaded.**
- **Oil on beaches should be mechanically removed using straw, peat moss, other highly absorbent material, or other appropriate techniques that will produce minimal deleterious effects on the biota.**
- **Failing recovery of oil from the sea surface or from a wrecked tanker, efforts should be made to burn it in place, provided the contamination is at a safe distance from shore facilities. If successful, this will minimize damage to the marine biota.**
- **Dispersants should be used only when necessary and should be of minimal potential toxicity to avoid even greater hazard to the environment.**
- **Sinking of oil is not recommended.**

All vessels using U.S. port facilities for the purpose of transporting oil or petroleum products should be required to demonstrate that effective procedures or devices, at least equivalent to the "Load on Top" procedure, are used to minimize oil releases associated with tank cleaning.

In order to protect marine wildlife:

- **recommendations listed above should be followed;**
- **a monitoring program should follow long-term trends in petroleum tar accumulation in selected areas of the oceans;**
- **no oil exploration or drilling should be permitted within existing or proposed sanctuaries, parks, reserves or other protected areas, or in their contiguous waters, in a manner which may deleteriously affect their biota;**

- **oil exploration or drilling should not be conducted in a manner which may deleteriously affect species subject to interstate or international agreements.**

TOXIC ORGANICS

The toxic organics constitute a considerable variety of chemical compounds, almost all of which are synthetic. The total production of synthetic organic chemicals in the U.S. in 1968 was 120,000 million pounds, a 15 per cent increase over 1967; 135,000 million pounds were produced in 1969, a 12 per cent increase over 1968 (United States Tariff Commission 1970).[377] This figure, in the order of 5×10^{13} grams, may be compared with the total productivity of the sea, which is in the order of 2×10^{16} grams of carbon incorporated into phytoplankton per year (Ryther 1969).[378] When considered in a global and future context, the production of synthetic chemicals by man cannot be considered an insignificant fraction of nature's productivity.

The majority of the synthetic organic chemicals, including those considered toxic, are readily degradable to elementary materials which reenter the chemical cycles in the biosphere. These pose no long-term hazard if applied or released into the environment in quantities sufficiently small to meet the recommendations for mixing zones (see p. 231).

The chemicals of most concern are the more stable compounds that enter the environment, whether they are introduced incidentally as waste materials or deliberately through their use. The toxicity, chemical stability, and resistance to biological degradation of such chemicals are factors that must be considered in assessing their potential effects on ecosystems. Moreover, because of the partitioning of nonpolar compounds among the components of marine ecosystems, relatively high concentrations of these, including halogenated hydrocarbons, are frequently found in organisms.

Only recently it was discovered that polychlorinated biphenyls (PCB), a class of chlorinated hydrocarbons used in a variety of industrial applications, were widespread contaminants in marine ecosystems (Duke et al.).[354a] Concentrations up to or higher than 1000 ppm in the body fat of estuarine birds have been recorded in both Europe and North America (Risebrough et al. 1968,[371] Jensen et al. 1969[363]). Moreover, both DDT and PCB have been found in organisms from depths of 3200 meters in the open North Atlantic Ocean (Harvey et al. 1972).[359]

The discovery of a man-made contaminant such as PCB, unknown in the environment a few years ago, in such unexpectedly high concentrations in marine organisms raises several questions. Are the concentrations of these compounds still increasing in the marine environment and at what rate, and what are the long-term effects upon the marine communities? Is it possible that other pollutants, undetected by the methodologies that measure the chlorinated hydrocarbons, are present in comparable amounts?

Criteria employed in the past to protect freshwater ecosystems were based on data now seen to be inadequate and on an approach that looked at pollutant concentrations in waste water effluents rather than in the receiving system. Evidently it is necessary to attempt to relate the amounts of input into the ecosystem to the levels in the various components of the ecosystem, including indicator organisms. The concentrations of a persistent pollutant in an indicator organism are considered the best way of following accumulation trends in an aqueous ecosystem that serves as a sink for the pollutant, once the capacity of the ecosystem to absorb the pollutant has been determined. If the concentrations in the indicator organisms exceed those considered safe for the ecosystem, input should then be reduced, restricted, or eliminated until environmental levels are acceptable on the basis of established criteria. Inputs of persistent pollutants into the marine environment, however, are in many cases indirect and not immediately controllable, e.g. river runoffs, atmospheric fallout, and dumping by foreign and domestic ships. The sources of the chemicals in atmospheric fallout may be located anywhere in the world.

Different recommendations must therefore be developed to protect the marine environment from increasing amounts and varieties of organic pollutants that might be anticipated over the next century. The same recommendations may be applied to estuaries, but these must also be protected from a variety of chemicals that are less persistent and pose no long-term hazard, but that may, because of toxic effects upon organisms, cause unacceptable amounts of damage. These include many of the pesticides, components of sewage, biological wastes from slaughter houses, and other organic wastes from industry.

Acute toxicity values and subacute effects of pesticides on marine life are listed in Appendix III–Table 6, and in Table IV–7, p. 265. Table IV–7 is a summary of the "most sensitive" organisms taken from Appendix III–Table 6 and includes a list of chemicals that are considered to have potential environmental importance in estuarine or marine ecosystems. The list includes many of the pesticides that are readily degradable in the environment but because of their high toxicity are potentially dangerous to estuarine ecosystems. The list, which should be revised as new data become available, proposes a minimum number of such chemicals. Appendix III–Table 6 includes the following information relative to the potential importance of each material as coastal and marine contaminants. (a) Production figures, which are taken from the 1969 Tariff Commission reports, are listed in the second column. The production figures provide a useful clue to the compounds that are of potential importance as marine pollutants. The order of the chemicals generally follows that of the Tariff Commission reports and is not intended to be a ranking in order of importance. (b) The third column of the table indicates

TABLE IV-7—Presence and Toxicity of Organic Chemicals in the Marine System

Chemical (1)	U.S. production pounds, gal./yr (2)	Presence in sea water or marine organisms (3)	Trophic accumulation (4)	Most sensitive organisms tested (5)	Formulation (6)	Conc. (ppb active ingredient in water) (7)	Method of assessment (8)	Test procedure (9)	Reference (10)
PESTICIDES, Total	1.1×10^{9} lb	...	...	...					
Fungicides									
Fungicides, total	1.4×10^{8}	...	...	...					
Pentachlorophenol	4.6×10^{7}	Expected	Unknown	Insufficient data for marine organisms					
2,4,5-Trichlorophenol	Not available (1969) 2.8×10^{7} (1968)	Unknown	Unknown	Crassostrea virginica American oyster	....	600	TLM	48 hr static lab bioassay	Davis and Hidu 1969
Nabam (Ethylene bis(dithiocarbamic acid), disodium salt)	1.9×10^{6}	Unlikely	Unlikely	Dunaliella tertiolecta	....	100	.270. O. D. expt/O.D. control	10 day growth test	Ukeles 1962
Hexachlorobenzene	Not available	Expected	Detected in birds (Vos et al., 1968) Koeman and Genderen, 1970)	Insufficient data for marine organisms					
Herbicides									
Herbicides, total	3.5×10^{8}	...	...	...					
Amitrole (3-amino-1,2,4-triazole)	Not available	Unlikely	Unlikely	Insufficient data					
Chloramben (3-amino-2,5-dichlorobenzoic acid, sodium salt)	Not available	Unlikely	Unlikely	Chlorococcum sp Phaeodactylum tricornutum	Methyl ester	2.5×10^{3}	50% decrease in growth	Growth measured as ABS. (525 mu) after 10 days	Walsh 1972
Picloram (4-amino-3,5,6-trichloropicolinic acid) (Tordon®)	Not available	Unlikely	Unlikely	Isochrysis galbana	....	1×10^{5}	50% decrease in O_2 evolution*	...	Walsh 1972
					....	5×10^{4}	50% decrease in growth	Measured as ABS (525 mu) after 10 dys	Walsh 1972
Simazine [2-chloro-4,6-bis-(ethylamino)-s-triazine]	Not available	Unlikely	Unlikely	Isochrysis galbana	Technical acid	500	50% decrease in growth	Measured as ABS. (525 mu) after 10 days	Walsh 1972
				Phaeodactylum tricornutum	Technical aid	500	50% decrease in growth	Measured as ABS (525 mu) after 10 days	Walsh 1972
Atrazine [2-chloro-4-ethylamino-6-isopropyl-amino-s-triazine]	Not available	Unlikely	Unlikely	Chlorococcum sp., Chlamydomonas sp., Monochrysis lutheri	Technical acid	100	50% decrease in growth	Measured as ABS. (525 mu) after 10 days	Walsh 1972
				Isochrysis galbana	Technical acid	100	50% decrease in O_2 evolution*		Walsh 1972
				Phaeodactylum tricornutum	Technical acid	100	50% decrease in O_2 evolution*		Walsh 1972
Monuron [3-(p-chlorophenyl)-1,1-dimethylurea]	Not available	Unlikely	Unlikely	Protococcus sp.	....	20	.00 OPT. DEN. expt opt DEN control	10 day growth test	Ukeles 1962
				Dunaliella tertiolecta	....	20	.00 OPT. DEN. expt opt DEN control	10 day growth test	Walsh 1972
				Phaeodactylum tricornutum	....	20	.00 OPT. DEN. expt opt DEN control	10 day growth test	Ukeles 1962
Diuron [3-(3,4,-dichlorophenyl)-1,1-dimethylurea]	Not available	Unlikely	Unlikely	Protococcus	....	0.02	.52 OPT. DEN. expt opt DEN control	10 day growth test	Ukeles 1962
				Monochrysis lutheri	....	0.02	.00 OPT. DEN. expt opt DEN control	10 day growth test	Ukeles 1962
Maleic hydrazide [1,2-dihydropyridazine-3,6-dione]	2.8×10^{6} lb.	Unlikely	Unlikely	Insufficient data					
Fenuron [1,1-dimethyl-3-phenylurea]	Not available	Unlikely	Unlikely	Chlorococcum sp.	Technical acid	750	50% decrease in growth	10 day growth test	Walsh 1972
				Isochrysis galbana	Technical acid	750	50% decrease in growth	10 day growth test	Walsh 1972
				Monochrysis lutheri	....	290	.67 OPT. DEN. expt opt DEN control	10 day growth test	Ukeles 1962
Ametryne [2-ethylamino-4-isopropylamino-6-methyl-mercapto-s-triazine]	Not available	Unlikely	Unlikely	Chlorococcum sp.	Technical acid	10	50% decrease in growth	Measured as ABS (525 mu) after 10 days	Walsh 1972
				Isochrysis galbana	Technical acid	10	50% decrease in O_2 evolution*		Walsh 1972
				Monochrysis lutheri Phaeodactylum tricornutum	Technical acid	10	50% decrease in O_2 evolution*		Walsh 1972

*, O_2 evolution measured by Gilson differential respirometer on 4 ml of culture in log phase. Length of test 80 minutes.

TABLE IV-7—Presence and Toxicity of Organic Chemicals in the Marine System—Continued

Chemical (1)	U.S. production pounds, gal, yr (2)	Presence in sea water or marine organisms (3)	Trophic accumulation (4)	Most sensitive organisms tested (5)	Formulation (6)	Conc. (ppb active ingredient in water) (7)	Method of assessment (8)	Test procedure (9)	Reference (10)
Herbicides, cont.									
Endothal [7-oxabicyclo-(2.2.1) heptane-2,3-dicarboxylic acid, disodium salt]	Not available	Unlikely	Unlikely	Mercenaria mercenaria Hard clam	....	1.25×10^{4}	TLM	12 day static lab bioassay	Davis and Hidu 1969[644]
MCPA [4-chloro-2-methylphenoxyacetic acid]	Not available	Unlikely	Unlikely	Crassostrea virginica American oyster	....	1.56×10^{4}	TLM	48 hr static lab bioassay	Davis and Hidu 1969[644]
2,4-D & derivatives	1.2×10^{8} lb	Unknown	Unknown	Crassostrea virginica American oyster Dunaliella tertiolecta	Ester	740	TLM	14 day static lab bioassay	Davis and Hidu 1969[644]
2,4,5-T & derivatives [2,4,5-trichlorophenoxyacetic acid]	2.8×10^{7} lb	Unknown	Unknown	Isochrysis galbana	Technical acid	5×10^{4}	50% decrease in O_2 evolution*	....	Walsh 1972[979]
				Phaeodactylum tricornutum	Technical acid	5×10^{4}	50% decrease in growth	Measured as ABS. (525 mu) after 10 days	Walsh 1972[979]
Silvex [2-(2,4,5-trichlorophenoxy)propionic acid]	1.6×10^{6}	Unlikely	Unlikely	Crassostrea virginica American oyster Dunaliella tertiolecta	....	710	TLM	14 day static lab bioassay	Davis and Hidu 1969[644]
Diquat [6,7-Dihydrodipyrido (1,2-a:2',1'-c)pyrazidiinium dibromide]	Not available	Unlikely	Unlikely	Chlorococcum sp.	Dibromide	5×10^{4}	50% decrease in O_2 evolution*	....	Walsh 1972[979]
				Dunaliella tertiolecta	Dibromide	5×10^{4}	50% decrease in O_2 evolution*	....	Walsh 1972[979]
				Isochrysis galbana	Dibromide	1.5×10^{4}	50% decrease in growth	Measured as ABS. (525 mu) after 10 days	Walsh 1972[979]
				Phaeodactylum tricornutum	Dibromide	5×10^{4}	50% decrease in O_2 evolution*	....	Walsh 1972[979]
Paraquat [1,1'-dimethyl-4,4'-dipyridinium dichloride]	Not available	Unlikely	Unlikely	Dunaliella tertiolecta	Dichloride	[illegible]	50% decrease in O_2 evolution*	....	Walsh 1972[979]
				Isochrysis galbana	Dichloride	5×10^{3}	50% decrease in growth	Measured as ABS. (525 mu) after 10 days	Walsh 1972[979]
Trifluralin [α,α,α-Trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine]	Not available	Unlikely	Unlikely	Chlorococcum sp.	Technical acid	2.5×10^{3}	50% decrease in growth	Measured as ABS. (525 mu) after 10 days	Walsh 1972[979]
				Isochrysis galbana	Technical acid	2.5×10^{3}	50% decrease in growth	Measured as ABS. (525 mu) after 10 days	Walsh 1972[979]
				Phaeodactylum tricornutum	Technical acid	2.5×10^{3}	50% decrease in growth	Measured as ABS. (525 mu) after 10 days	Walsh 1972[979]
Cacodylic acid [Hydroxydimethyl arsine oxide]	Not available	Unlikely	Unlikely	Insufficient data					
Insecticides									
Insecticides, total (includes rodenticides)	5.7×10^{8} lb								
Heptachlor [Heptachloro-tetrahydro-endo-methano-indene] (includes heptachlor epoxide)	Not available	Oysters (Bugg et al. 1967)[640]	Bald Eagles (Krantz et al. 1970)[665]	Thalassoma bifasciatum Bluehead	100%	0.8	LC-50	96 hr static lab bioassay	Eisler 1970b[647]
Endrin [Hexachloro-epoxy-octahydro-endo-endo-dimethanonaphthalene]	Not available	Oysters (Bugg et al. 1967,[640] Casper, 1967,[333] Rowe et al. 1971)[973]	Brown Pelican (Schreiber and Risebrough 1972,[974] Risebrough et al. 1968)[371]	Mugil cephalus Striped mullet	100%	0.3	LC-50	96 hr static lab bioassay	Eisler 1970b[647]
				Menidia menidia Atlantic silverside	100%	0.05	LC-50	96 hr static lab bioassay	Eisler 1970b[647]
Dieldrin [Hexachloro-epoxy-octahydro-endo-exo-dimethanonaphthalene]	Not available	Oysters (Bugg et al. 1967,[640] Casper, 1967,[333] Rowe et al. 1971)[973]	Bald eagles (Krantz et al. 1970)[665] Grey Whale, Sperm Whale (Wolman and Wilson 1970)[980] Brown Pelican (Schreiber and Risebrough 1972)[974]	Anguilla rostrata American eel	100%	0.9	LC-50	96 hr static lab bioassay	Eisler 1970b[647]
Aldrin [Hexachloro-hexahydro-endo-exo-dimethanonaphthalene]	Not available	Oysters (Bugg et al. 1967)[640]	Unlikely, converts to dieldrin (Korschgen 1970)[664]	Palaemon macrodactylus Korean shrimp	Technical	0.74 (0.51-1.08)	TL-50	96 hr static lab bioassay	Earnest (unpublished)[893]
Chlordane [Octachloro-tetrahydro-methanoindene]	Not available	Oysters (Bugg et al. 1967)[640]	Expected	Palaemon macrodactylus Korean shrimp	100%	10 (10-30)	TL-50	96 hr static lab bioassay	Earnest (unpublished)[893]

* O_2 evolution measured by Gilson differential respirometer on 4 ml of culture in log phase. Length of test 90 minutes.

TABLE IV-7—Presence and Toxicity of Organic Chemicals in the Marine System—Continued

Chemical (1)	U.S. production pounds, gal./yr (2)	Presence in sea water or marine organisms (3)	Trophic accumulation (4)	Most sensitive organisms tested (5)	Formulation (6)	Conc. (ppb active ingredient in water) (7)	Method of assessment (8)	Test procedure (9)	Reference (10)
Insecticides, cont.									
Strobane[R] (polychlorinated terpenes)	Not available	Expected	Expected	Insufficient data for marine species					
Toxaphene (Chlorinated camphene)	Not available	Bay mussel (Madin, 1968);[967] Oysters (Bugg et al. 1967)[340]	Expected	Gasterosteus aculeatus threespine stickle-back	100%	7.8	TLM	96 hr static lab bioassay	Katz 1961[illegible]
DDT compounds	1.2×10[8] lb.	Jensen et al. 1969,[369] Risebrough et al. 1968[271]							
p,p'-DDT [1,1,1-Trichloro-2,2-bis(p-chlorophenyl) ethane	...	(References cited above)		Penaeus duorarum Pink shrimp	Technical 77%	0.12 0.17 (0.09–0.32)	TL-50 TL-50	28 day bioassay 96 hr intermittent flow lab bioassay	Nimmo et al. 1970[illegible] Earnest (unpublished)[259]
p,p'-DDD(p,p'-TDE) [1,1-Dichloro-2,2-bis (p-chlorophenyl)ethane	...			Palaemon macrodactylus	99%	2.5 (1.6–4.0)	TL-50	96 hr intermittent flow lab bioassay	Earnest (unpublished)[259]
p,p'-DDE [1,1-Dichloro-2,2-bis(p-chlorophenyl) ethylene	....	(References cited above)		Falco peregrinus Peregrine Falcon	....	....	Eggshell thinning	DDE in eggs highly correlated with shell thinning	Cade et al. 1970[351]
Mirex [Dodecachloro-octahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalene]	Not available	Expected	Expected	Penaeus duorarum Pink shrimp	Technical	1.0	100% paralysis/death in 11 days	Flowing water bioassay	Lowe et al. 1971[illegible]
Benzene hexachloride [Hexachlorocyclohexane]	Not available	Southern hemisphere sea birds (Tatton and Ruzicka 1967)[376]		Penaeus setiferus White shrimp	0.1%	2.8	TLM	24 hr static lab bioassay	Chin and Allen 1958[353]
Lindane [gamma-hexachlorocyclohexane]	Not available	Oysters (Bugg et al. 1967,[340] Casper 1967)[363]	Expected Sand shrimp	Crangon septemspinosa Sand shrimp Pagurus longicarpus Hermit crab	100% 100%	5 5	LC-50 LC-50	96 hr static lab bioassay 96 hr static lab bioassay	Eisler 1969[355] Eisler 1969[355]
Endosulfan [Hexachlorohexahydro-methano-benzo-dioxathiepin-3-oxide] (Thiodan[R])	Not available	Bay mussel (Koeman and Genderen 1970)[440]	Sandwich Tern, Common Eider (Koeman and Genderen 1970)[443]	Palaemon macrodactylus Korean shrimp	89%	3.4 (1.6–6.3)	TL-50	96 hr intermittent flow lab bioassay	Earnest (unpublished)[259]
Methoxychlor [1,1,1-Trichloro-2,2-bis(p-methoxy-phenyl)ethane]	Not available	Oysters (Bugg et al. 1967)[340]	Unlikely	Palaemon macrodactylus	89.5%	0.44 (0.21–0.93)	TL-50	96 hr static lab bioassay	Earnest (unpublished)[392]
Carbaryl (Sevin) [1-naphthyl-N-methylcarbamate]	Not available	Unlikely	Unlikely	Palaemon macrodactylus	100%	7.0 (1.5–28)	TL-50	96 hr intermittent bioassay	Earnest (unpublished)[392]
				Cancer magister Dungeness crab	80%	6	Prevention of hatching and molting	24 hr static lab bioassay	Buchanan et al. 1970[349]
Coumaphos (Co-ral) [O,O-Diethyl-O-(3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl)-phosphorothioate]	Not available	Unlikely	Unlikely	Crassostrea virginica American oyster	....	110	TLM	48 hr static lab bioassay	Davis and Hidu 1969[354]
Diazinon [O,O-Diethyl-O-(2-isopropyl-4-methyl-6-pyrimidinyl)phosphorothioate]	Not available	Unlikely	Unlikely	Insufficient data					
Parathion [O,O-Diethyl-O-p-nitrophenyl-phosphorothioate]	Not available	Unlikely	Unlikely	Cyprinodon variegatus Sheepshead minnow	....	10	Acetylcholinesterase activity in control vs. expt groups. Control = 1.36; Expt. = 0.120	72 hr static exposure	Coppage (unpublished)[361]
Dursban [O,O Diethyl-O-3,5,6-trichloro-2-pyridyl-phosphorothioate]	Not available	Unlikely	Unlikely	Palaemon macrodactylus Korean shrimp	....	0.01 (0.002–0.046)	TL-50	96 hr intermittent flow bioassay	Earnest (unpublished)[392]
Fenthion [O,O-Dimethyl-O-(4-methylthio-m-tolyl) phosphorothioate] (Baytex)	Not available	Unlikely	Unlikely	Palaemon macrodactylus	....	3.0 (1.5–60)	TL-50	96 hr intermittent flow bioassay	Earnest (unpublished)[392]
Methyl parathion [O,O,-Dimethyl-O-p-nitrophenyl-phosphorothioate]	5.1×10[7] lb	Unlikely	Unlikely	Crangon septemspinosa Sand shrimp	100%	2	LC-50	96 hr static lab bioassay	Eisler 1969[355]
Guthion [O,O-Dimethyl-S-(4-oxo-1,2,3-benzotriazino-3-methyl)phosphorodithioate]	Not available	Unlikely	Unlikely	Gasterosteus aculeatus threespine stickle-back	93%	4.8	TLM	96 hr static lab bioassay	Katz 1961[illegible]
Dioxathion (Delnav) [2,3-p-Dioxane-S,S-bis(O,O-diethylphosphorodithioate]	Not available	Unlikely	Unlikely	Crangon septemspinosa Sand shrimp Fundulus heteroclitus Mummichog Menidia menidia Atlantic silverside	100% 100% 100%	38 6 6	LC-50 LC-50 LC-50	96 hr static lab bioassay 96 hr static lab bioassay 96 hr static lab bioassay	Eisler 1969[355] Eisler 1970a[356] Eisler 1970b[357]

TABLE IV-7—Presence and Toxicity of Organic Chemicals in the Marine System—Continued

Chemical (1)	U.S. production pounds, gal. yr (2)	Presence in sea water or marine organisms (3)	Trophic accumulation (4)	Most sensitive organisms tested (5)	Formulation (6)	Conc. (ppb active ingredient in water) (7)	Method of assessment (8)	Test procedure (9)	Reference (10)
Insecticides, cont.									
Phosdrin [1-methoxycarbonyl-1-propen-2-yl dimethylphosphate]	Not available	Unlikely	Unlikely	Crangon septemspinosa Sand shrimp	100%	11	LC-50	96 hr static lab bioassay	Eisler 1969[244]
Malathion [S-(1,2-dicarbethoxyethyl)-O,O-dimethylphosphorodithioate]	Not available	Unlikely	Unlikely	Thalassoma bifasciatum Bluehead	100%	27	LC-50	96 hr static lab bioassay	Eisler 1970b[247]
Phosphamidon [2-Chloro-N,N-diethyl-3-hydroxycrotonamide dimethyl phosphate]	Not available	Unlikely	Unlikely	Insufficient data					
Phorate [O,O Diethyl-S-[(Ethylthio)methyl]-phosphorodithioate]	Not available	Unlikely	Unlikely	Cyprinodon variegatus Sheepshead minnow	....	5	Acetylcholinesterase activity** in control vs expl. groups. Control=1.36; Expt.=0.086	72 hr static exposure	Coppage (unpublished)[236]
DDVP [O,O-Dimethyl-O-(2,2-dichlorovinyl)phosphate]	Not available	Unlikely	Unlikely	Crangon septemspinosa Sand shrimp	....	4	LC-50	96 hr static lab bioassay	Eisler 1969[244]
Trichlorfon [O,O-Dimethyl-1-hydroxy-2,2,2-trichloroethylphosphonate] (Dipterex)	Not available	Unlikely	Unlikely	Crassostrea virginica American oyster	....	1,000	TLM	48 hr static lab bioassay	Davis and Hidu 1969[234]
TEPP [Tetraethyl pyrophosphate]	Not available	Unlikely	Unlikely	Crassostrea virginica	....	$>1\times10^{4}$	TLM	14 day static lab bioassay	Davis and Hidu 1969[234]
Related products									
DBCP [1,2-Dibromo-3-chloropropane] (Nemagon[R])	8.6×10^{6} lb	Unknown	Unknown	Mercenaria mercenaria Hard clam	....	700	TLM	12 day static lab bioassay	Davis and Hidu 1969[234]
Methyl bromide	2.0×10^{7} lb	Unknown	Unknown	Insufficient data					
TAR AND TAR CRUDES									
Benzene	1.2×10^{9} gal.	Unknown	Unknown	Insufficient data					
Toluene	7.6×10^{8} gal.	Unknown	Unknown	Insufficient data					
Xylene	3.8×10^{8} gal.	Unknown	Unknown	Insufficient data					
Naphthalene	8.5×10^{8} gal.	Unknown	Unknown	Insufficient data					
PLASTICIZERS									
Phthalic anhydride esters, total	8.8×10^{8} lb.	Expected	Unknown	Insufficient data					
Adipic acid esters, total	6.6×10^{7}	Unknown	Unknown	Insufficient data					
SURFACE-ACTIVE AGENTS									
Dodecylbenzenesulfonates, total	5.7×10^{8} lb. (1968)	Unknown	Unlikely	Insufficient data					
Lignosulfonates, total	4.4×10^{8} lb.	Unknown	Unknown	Insufficient data					
Nitrilotriacetic acid	Not available	Unknown	Unlikely	Cyclotella nana	Monohydrated sodium salt	5×10^{5}	38% growth as compared to controls	72 hr static lab bioassay	Erickson et al. 1970[249]
				Homarus americanus American lobster	Monohydrated sodium salt	1×10^{5}	100% mortality	7 day static lab bioassay	NMWQL 1970[300]
HALOGENATED HYDROCARBONS									
Carbon tetrachloride	7.6×10^{8} lb (1968)	Unknown	Unlikely	Insufficient data					
Dichlorodifluoromethane ...	3.3×10^{8} (1968)	Unknown	Unlikely	Insufficient data					
Ethylene dichloride	4.8×10^{9} (1969)	Expected	Unlikely	Insufficient data					
Aliphatic chlorinated hydrocarbon wastes of vinyl chloride production	3×10^{7} lb (estimated as 1% of vinyl chloride production)	Surface waters and marine organisms of North Atlantic and North Sea (Jensen et al. 1970)[261]	Unknown	Gadus morrhua Cod	67% 1,1,2-trichloroethane, 30% 1,2-dichloroethane	10,000	LC-50	10 hr lab bioassay	Jensen et al. 1970[261]
Polychlorinated biphenyl ...	Not available	Jensen et al. 1969[260], Risebrough et al. 1968[271]	....	Penaeus duorarum Pink shrimp	Aroclor 1254	0.94	51% mortality	15 day chronic exposure in flowing seawater	Nimmo et al. 1971[329]
Polychlorinated terphenyl	Not available	Expected	Expected	Insufficient data					
Brominated biphenyls	Not available	Unknown	Expected	Insufficient data					
CYCLIC INTERMEDIATES									
Monochlorobenzene	6.0×10^{8} lb	Expected	Unlikely	Insufficient data					
Phenol	1.7×10^{9} lb	Expected	Unlikely	Mercenaria mercenaria Hard Clam	....	5.3×10^{4}	TLM	48 hr static lab bioassay	Davis and Hidu 1969[234]
MISCELLANEOUS CHEMICALS									
Tetraethyl lead	4.9×10^{8}	Unlikely	Unlikely	Insufficient data					

** ACh hydrolyzed, hr/mg brain.

whether or not the compound has been detected in sea water or in marine organisms. Compounds which have been detected are of greater immediate concern than those which have not. Frequently, because of their low solubility in water, some of the non-polar compounds which are biologically accumulated can be detected in an organism but not in the water itself. (c) The fourth column, trophic accumulation, indicates whether the compound has been shown to pass through the food web from prey species to predator. Compounds that are so accumulated are of greater concern than compounds of comparable toxicity which are not. Finally, the species thought to be most sensitive to the compound are indicated in the final columns with reference to original studies in the scientific literature. These data are useful as a guide only and are not sufficient in themselves for definitive evaluation of the environmental significance of each compound.

The report, "The Effects of Chemicals on Aquatic Life, vol. 3, Environmental Protection Agency, Water Quality Office, 1971," has been useful as a guide to the available toxicity data of industrial chemicals on marine organisms. Appendix III–Table 6 is a compendium of data on toxicity of pesticides to marine organisms. These sources are incomplete and should be continually revised.

Bases for Recommendations

1. In order to provide an adequate level of protection for commercially important marine species and for species considered important in the maintenance of stability of the ecosystem, an application factor of one one-hundredth (0.01) is used when pesticides or organic wastes that are not trophically accumulated in food webs are applied or released in estuarine or marine environments. This factor is arbitrary and was derived from data available on marine and freshwater organisms. (See Section III, p. 121.) It assumes that a concentration of one one-hundredth (0.01) of that causing harm to the most sensitive species to be protected will not damage this species or the ecosystem. Future studies may show that the application factor must be decreased or increased in magnitude.

2. The application factor may also be used for the compounds that are trophically accumulated in food webs in order to protect fish and invertebrates to which these compounds are toxic. It cannot be used, however, to protect fish-eating birds and mammals which may trophically accumulate these compounds from their prey species, in part because sublethal effects such as eggshell thinning and hormone imbalance may adversely affect reproductive capacity and therefore the long term survival of populations. Levels that would protect fish-eating birds and mammals against the effects of compounds that are trophically accumulated from prey species are given in the discussion of Marine Wildlife (see pp. 224–228).

The recommendations below apply to all organics of both proved and potential toxicity.

Recommendations

In general, marine life with the exception of fish-eating birds and mammals should be protected where the maximum concentration of the chemical in the water does not exceed one one-hundredth (0.01) of the LC50 values listed in Column 7, Table IV–7, pp. 265–268. If new data indicate that an ecosystem can adequately degrade a particular pollutant, a higher application factor for this pollutant may be used.

In order to maintain the integrity of the ecosystem to the fullest possible extent, it is essential to consider effects on all non-target organisms when applying pesticides to estuarine habitats in order to control one or more of the noxious species. For those occasions when chemicals must be used, the following guidelines are offered:

- **a compound which is the most specific for the intended purpose should be preferred over a compound that has broad spectrum effects;**
- **a compound of low persistence should be used in preference to a compound of greater persistence;**
- **a compound of lower toxicity to non-target organisms should be used in preference to one of higher toxicity;**
- **water samples to be analyzed should include all suspended particulate and solid material: residues associated with these should therefore be considered as present in the water;**
- **when a derivative such as p.p′-DDE or 1-napthol is measured with or instead of the parent compound, the toxicity of the derivative should be considered separately: if the toxicity of a derivative such as an ionic species of a pesticide is considered equivalent to that of the original parent compound, concentrations should be expressed as equivalents of the parent compound.**

It is recommended that the chemicals listed in Table IV–7 and all chemicals subsequently added to this list be considered as toxic organic compounds potentially harmful to the marine environment. It is emphasized that the data in Table IV–7 are not sufficient in themselves for final evaluation of the environmental significance of each compound.

OXYGEN

An extensive review and discussion of the present information on biological responses to variations in dissolved oxygen has been published recently by Doudoroff and

Shumway (1970).[312] This review has been used in developing oxygen recommendations by both the Freshwater and Marine Panels in their reports. On the basis of this large body of information, recommendations for "levels of protection" for freshwater fish populations have been developed. Estuarine and marine organisms have not been studied as extensively, and the present information is inadequate for satisfactory analysis of the response of communities to temporal and spatial variations in dissolved oxygen concentrations.

The generalizations presented by the Freshwater Panel appear to be valid, with qualifications, for estuarine and marine situations.

1 A reduction in dissolved oxygen concentration reduces the rate of oxygen uptake by aquatic plants and animals. However, as noted by Doudoroff and Shumway, the observed response of many organisms under laboratory conditions measured in such terms as growth rate, swimming speed, or hatching weight, shows fractional or percentage reductions that approximately correlate with the logarithm of the deviation of the dissolved oxygen concentration from equilibrium with the atmosphere, under conditions of *constant* dissolved oxygen concentrations. Thus, reduction in the dissolved oxygen concentration by 1 mg/l from the saturation value has much less effect than reduction by 1 mg/l from the 50 per cent of saturation value.

2 The non-threshold character of these responses means that some risk of effect on the aquatic populations is associated with any reduction in the dissolved oxygen concentrations. As noted above, the risk of damage increases as dissolved oxygen concentrations decrease from saturation values. Selection of risk acceptance is a social and economic evaluation involving other uses of any particular environment that must precede recommendations derived using the risk acceptance and the pertinent scientific information.

3 Consideration of the effects of dissolved oxygen concentrations on aquatic life must include the responses of developing eggs and larvae, as well as the maturing and adult individuals. Species that have limited spawning areas should be identified and the biological risk of decreased oxygen concentrations evaluated accordingly.

For estuaries and coastal waters, consideration must be given to the distribution of dissolved oxygen with depth, since even under natural conditions low oxygen concentrations may be found in the deeper waters. Special consideration should be given to estuary type, topography, currents, and seasonal development of pycnoclines.

Many estuaries and coastal regions are highly productive, and the characteristic pattern with photosynthesis in the upper-water layer or adjacent marshes leads to large population densities in the upper layers and loss of oxygen to the atmosphere from the supersaturated surface waters or the marsh plants. Subsequent decomposition of these organisms and their wastes in the deeper waters leads to oxygen depletion. Several deeper coastal plain estuaries and fjords show oxygen depletion from this sequence. Addition of mineral and organic plant nutrients to such regions may intensify the production and subsequent decomposition processes. The effects of particular additions will depend on the water depths and rate of vertical mixing, and it is necessary to construct an oxygen balance model for each case. Sewage treatment that consists of partial or nearly complete mineralization of the organic materials may still produce a discharge that will damage the aquatic system, i.e., an amount of organic matter nearly equal in oxygen demand to the original sewage is produced in the environment. The principal effect of many "secondary" treatment systems is the trading of an intense local effect near the outfall for a more widespread effect at greater distances. One of the major considerations in defining water quality recommendations for nutrients in any estuarine or coastal region should be the risk associated with oxygen depletions from increased production. Deliberate moderate additions of nutrients to increase the yield of some fishery should also give due regard to this secondary effect.

Recommendation

Each proposed change in the dissolved oxygen concentration in estuaries and coastal waters should be reviewed for risk of damage to aquatic life. The limited laboratory data and field observations on marine organisms suggest that easily observed effects, which are in many cases deleterious, occur with dissolved oxygen concentrations of 4 to 5 mg/l as daily minimum values for periods of several days. As a guideline, therefore, reduction of the dissolved oxygen concentration to values below 4 mg/l can be expected to change the kinds and abundances of the aquatic organisms in the affected volume of water and area of bottom. Particular attention should be directed toward identifying species with restricted spawning and nursery areas and conservatism should be used in applying guidelines to these areas. (See the expanded discussion in Section III, pp. 131–135.)

RADIOACTIVE MATERIALS IN THE AQUATIC ENVIRONMENT

This section considers radioactivity in all surface waters inhabited by plants and animals including fresh, estuarine, and marine waters of the U.S. The subject matter pertains primarily to the impact of environmental radioactivity on aquatic organisms, although it also contains some discussion of human radiation exposure from aquatic food chains. A recent report by the National Academy of Sciences (1971)[397] presented a review of radioactivity in the marine environment, and that review has been used extensively in the preparation of this report.

Characteristics and Sources of Radioactivity

Radiation is the energy emitted spontaneously in the process of decay of unstable atoms of radioisotopes. This energy can exist either in the form of electromagnetic rays or subatomic particles and cannot be detected by man's senses. Radiation can be detected, however, by means of electronic instruments, and quantities present at very low levels in the environment can be measured with remarkable accuracy. Radioactivity which occurs naturally in the environment originates from primordial radioisotopes and their decay products (daughters) and from reactions between cosmic rays from outer space and elements in the atmosphere or in the earth. Some of the more abundant primordial radioisotopes in terms of their radioactivity are potassium (^{40}K), palladium (^{234}Pd), rubidium (^{87}Rb), uranium (^{238}U) and thorium (^{232}T), the first accounting for 90 per cent of the natural radiation in the oceans. While beryllium (^{7}Be) is the most abundant radioisotope produced by cosmic rays, carbon (^{14}C) and hydrogen (^{3}H) (tritium) are biologically the most interesting. The presence of natural radioactivity was unknown until 1896 when Becquerel discovered uranium. Until the development of the atomic bomb during World War II, virtually all of the radioactivity on earth came from natural sources.

The first man-made radioisotopes were not released into the environment in any significant amounts until the atomic bomb was tested and used in war even though the uranium 235 atom was first split (fissioned) by neutron bombardment in 1938. While the release of radioisotopes was drastically reduced with the halting of nuclear weapons testing in the atmosphere by signatories of the test ban treaty, radioactive wastes continue to be released from nuclear powered ships and submarines, nuclear power plants, nuclear fuel reprocessing plants, and to a lesser extent from laboratories and hospitals. Two methods have been used in handling radioactive wastes. High levels have been concentrated and held in special storage tanks, while low levels of radioactive wastes in small volumes have been diluted and dispersed in the aquatic environment—particularly in the oceans. Some manmade radioisotopes, such as strontium 90 and cesium 137, are the debris of split atoms and are called fission products. Other radioisotopes, such as zinc 65 and cobalt 60, are activation products, produced when stray neutrons from the fission process strike the atoms of stable elements.

Cycling of Radioactive Materials The physical, chemical, and physiological behavior of radioisotopes is essentially identical with that of the stable isotopes of the same element—at least until disintegration occurs. It should be pointed out, however, that in some instances the physical and chemical states of a radioisotope introduced into the aquatic environment may vary from that of the stable element in water. At the time of disintegration, the decaying atoms change into different types of atoms of the same element or into atoms of a different element. If the behavior of a particular element in an ecosystem is known, the behavior of the radioisotopes of that element can be predicted. The reverse also is true, and radioisotopes can serve as excellent tracers in following the movement of elements through complex environmental systems. Radioactive wastes in the aquatic environment may be cycled through water, sediment, and the biota. Each radioisotope tends to take a characteristic route and has its own rate of movement through various temporary reservoirs. The route taken by tritium is different from that of other radioisotopes. Tritium becomes incorporated in the water molecule and cannot be removed by present waste treatment practices. It is not concentrated appreciably by either biota or sediments.

When radioactive materials enter surface waters they are diluted and dispersed by the same forces that mix and distribute other soluble or suspended materials (National Academy of Sciences 1957).[293] The dominant forces are mechanical dilution that mixes radioisotopes in the waste stream as it leaves an outfall structure; advection and turbulent diffusion that mix materials in the receiving waters; and major transport currents that move masses of water over relatively long distances. On the other hand, precipitation and sedimentation tend to restrict the area of dispersion. When first introduced into fresh or marine water, a substantial part of the materials present in radioactive wastes becomes associated with solids that settle to the bottom, and many of the radioisotopes are bound chemically to the sediments. The sediments may also be moved geographically by currents. Even though in some instances sediments remove large quantities of radioisotopes from the water, and thus prevent their immediate uptake by the biota, this sediment-associated radioactivity may later leach back to the water and again become available for uptake by the biota.

Plants and animals, to be of any significance in the passage of radioisotopes through a food web in the aquatic environment, must accumulate the radioisotope, retain it, be eaten by another organism, and be digested. Radioisotopes may be passed through several trophic levels of a food web, and concentrations can either increase or decrease from one trophic level to the next, depending upon the radioisotope and the particular prey-predator organisms. This variation among trophic levels occurs because different organisms within the same trophic level have different levels of concentration and different retention times, which depend upon their metabolism or capacity to concentrate a given radioisotope. The concentration of a radioisotope by an organism is usually discussed in terms of a concentration factor: the ratio of the concentration of the radioisotope in the organism to that in its source, that is, the amount in water or food. Radioisotopes with short half-lives are less likely to be highly concentrated in the higher trophic levels of the food chain because of the time required to move from the water to plants, to herbivores, and eventually to carnivores. Organisms that concentrate radioisotopes to a high level and retain them for long periods of time have been referred to as "biological indicators for radioactivity." These organisms are of value in showing the presence of radioactive materials even though the concentrations in the water may be less than detectable limits.

Exposure Pathways

The radiation emitted by radioisotopes that are present in aquatic ecosystems can irradiate the organisms in many different ways. In order to evaluate the total radiation dose received by the aquatic organisms, and thus the risk of their being injured, all sources of exposure must be considered. These sources include both natural and man-made radiation, both external and internal.

Major Sources of External Radiation 1 Radioisotopes in the surrounding water that tend to remain in solution, or at least suspended in the water, become associated more readily with aquatic organisms than the radioisotopes that settle out.

2 Radioisotopes present on or fixed to sediments are significant to aquatic life, particularly to benthic organisms in the vicinity of existing major atomic energy plants.

3 Radioisotopes attached to the outer surfaces of organisms are of greater significance to micro-organisms, which have a larger surface-to-volume ratios, than shellfish or fish.

4 Cosmic-rays are of relatively minor importance to aquatic life that lives a few feet or more below the water surface, because of the shielding afforded by the water.

Major Sources of Internal Radiation 1 Radioisotopes in the gastrointestinal tract frequently are not assimilated, but during their residence in the tract expose nearby internal organs to radiation.

2 Assimilated radioisotopes are absorbed from water through the integument or from food and water through the walls of the gastrointestinal tract, metabolized, and are incorporated into tissues where they remain for varying periods of time. Aquatic plants, including algae absorb radioactive materials from the ambient water and from the interstitial water within the sediments.

It is difficult to measure the amount of radiation absorbed by aquatic organisms in the environment because they are simultaneously irradiated by radioisotopes within their body, on the surface of their body, in other organisms, in the water, and in sediments. Exposure thus depends on an organism's position in relation to the sediments and to other organisms, and to movement of some species in and out of the contaminated area.

Biological Effects of Ionizing Radiation

Ionizing radiation absorbed by plant and animal tissue may cause damage at the cellular and molecular levels. The degree of radiation damage to an organism depends upon the source (external or internal), the type (electromagnetic or particulate), the dose rate (intensity per unit of time), and the total dose. Possible effects to the individual organism may include death, inhibition or stimulation of growth, physiological damage, changes in behavioral patterns, developmental abnormalities, and shortening of life span. In addition, the extent of biological damage from radiation can be modified by environmental stresses such as changes in temperature and salinity. Under certain conditions, irradiation can cause gross pathological changes which are easily observed, or more subtle changes which are difficult or impossible to detect. In addition to somatic changes which affect the individual, genetic changes also may occur which may affect the offspring for many generations. At one time, it was widely believed that there was a threshold radiation dose below which damage did not occur, but now the consensus of most radiobiologists is that any increase over background radiation will have some biological effect. While the non-existence of a threshold dose is difficult to prove, most radiation biologists agree that even background levels of radiation from primordial radioisotopes and cosmic rays have resulted in some genetic changes over the ages. These radiation-induced changes usually constitute less than 1 per cent of all spontaneously occurring mutations (Asimov and Dobzhansky 1966).[344]

The amount of radiation absorbed by an organism can be expressed in various ways. The rad (radiation absorbed dose) is the unit used to measure the absorbed dose of radiation and refers to the absorption of 100 ergs of energy per gram of irradiated material. Because a rad of alpha or neutron radiation produces greater biological damage than a rad of gamma radiation, another unit called the rem (roentgen equivalent man) also is used. To obtain the rem, or dose equivalent, the number of rads absorbed by the tissue is multiplied by the quality factor and other necessary modifying factors to compensate for the effects of different types of radiation. The acute doses of radiation required to produce somatic damage to many species of aquatic organisms have been established within broad limits (National Academy of Sciences 1971).[337] Some bacteria and algae can tolerate doses of many thousands of rads, but the mean lethal dose (LD^{50}—30 days) for fish is in the range of several hundred to a few thousand rads. Eggs and early developmental stages are more sensitive than are adults. By comparison, the mean lethal dose for humans is about 300 rads.

The acute mean lethal dose has little value in placing restrictions on the amounts of radioactive material present in aquatic environments. Much more meaningful is the highest level of chronic exposure that results in no demonstrable damage to aquatic populations. A vast amount of research on dose-effect relationships for warm-blooded animals has led to the recommendations on human radiation exposure. People who work with radiation may receive no more than 5 rem in any one year. The recommended limit for the general public is 0.5 rem in one year for individuals but is restricted to only 0.17 rem per year as an average for populations. The lower level permitted for populations is to reduce the possibility of genetic changes becoming established.

Compared with the experimental data available for warm-blooded animals, only a meager amount of information is available on chronic dose-effect relationships for aquatic forms. The preponderance of available data indicates, however, that no effects are discernible on either individual aquatic organisms or on populations of organisms at dose rates as high as several rads per week. In populations of wild species, genetic damage may be removed by natural selection and somatically weakened individuals are probably eaten by predators. Consequently, aquatic organisms adversely affected by radiation are not readily recognized in the field.

The natural populations of fish that have probably sustained the greatest exposure to man-made radioactive materials are those near major atomic energy installations, for example, in the Columbia River near Hanford; in White Oak Creek and White Oak Lake, near Oak Ridge; and in the Irish Sea near Windscale, England. Small fish which received chronic irradiation of about 10.9 rads per day from radioisotopes in the sediments of White Oak Creek produced larger broods but with a higher incidence of abnormal embryos (Blaylock and Mitchell 1969).[385] Chironomid larvae living in the bottom sediments and receiving about five rads per week had an increased frequency of chromosomal aberrations but the abundance of the worms was not affected. The stocks of plaice in the vicinity of the Windscale outfall have been unaffected by annual dose rates of about 10 rads per year—primarily from the bottom sediments (Ministry of Agriculture, Fisheries and Food 1967).[392] Columbia River salmon spawning in the vicinity of the Hanford outfalls have been unaffected by doses in the range of 100 to 200 millirads per week (Watson and Templeton *in press*).[402] These observations on chronic exposure of aquatic organisms provide a subjective assessment of radiation sensitivities in natural populations but are not sufficiently definitive to form the basis for the development of water quality recommendations.

Restrictions on Radioactive Materials

The amounts of radioactive materials present in water must be restricted in order to assure that populations of organisms are not damaged by ionizing radiation and also to limit the amount of radioactive material reaching man via aquatic food chains. Permissible rates of intake of the various radioisotopes by man have been calculated so that the resulting annual dose is no greater than the recommended limit. Therefore, when the rate of consumption of aquatic organisms is determined, e.g., pounds of fish or shellfish per year, maximum concentrations of radionuclides permissible in the edible parts of the organisms can be computed. These maximum concentrations are well below the concentrations which have produced detectable effects on natural aquatic populations. It is probable that the aquatic environment will be protected by the restrictions currently imposed on the basis of human health.

The regulations which serve to protect man from radiation exposure are the result of years of intensive studies on the biological effects of radiation. Vast amounts of information have been considered by the International Commission on Radiological Protection (ICRP) (1960,[389] 1964,[390] 1965[391]), the National Council on Radiation Protection and Measurements (NCRP) (1959,[397] 1971[398]), and the U.S. Federal Radiation Council (FRC) (1960,[387] 1961[388]), in developing recommendations on the maximum doses of radiation that people may be allowed to receive under various circumstances or that may occur in water. The Drinking Water Standards (U.S. Department of Health, Education and Welfare, Public Health Service 1962[400]) and the Code of Federal Regulations (1967)[386] are responsive to the recommendations of the FRC, ICRP, and NCRP, and provide appropriate protection against unacceptable radiation dose levels to people where drinking water is the only significant source of exposure above natural background. Where fish or other fresh or marine products that have accumulated radioactive materials are used as food by humans, the concentrations of the radioisotopes in the water must be further restricted to ensure that the total intake of radioisotopes from all sources will not exceed the recommended levels.

Conclusions

Previous attempts to restrict radioactive discharges to marine environments have resulted in recommended maximum permissible concentrations in sea water (National Academy of Sciences 1959a,[393] 1959b,[394] 1962,[395] 1971[396]). These recommendations are most useful as a first approximation in predicting safe rates of discharge of radioactive wastes, but their applicability as water quality recommendations is limited and they are not intended for general use in fresh or estuarine waters where the concentrations of a great variety of chemical elements vary widely.

Three approaches to the control of levels of radioactivity in the aquatic environment have been used: (1) controlling the release of radioactivity based upon the specific activity approach—the ratio of the amount of radioactive isotope present to the total amount of the element (microcuries per milligram) (National Academy of Sciences 1962),[395] (2) relating the effects of radiation upon aquatic organisms caused by a given concentration of a radioisotope or combinations of radioisotopes in the water, and (3) restricting concentrations of radioisotopes to those permitted in water and food for human consumption.

Since concentrations of stable elements vary from one body of water to another, and with time, and since adequate data are not available to relate effects of radiation upon aquatic organisms to specific levels of radioactivity in the water, restrictions contained in the Code of Federal Regulations (1967)[396] on liquid effluents are considered adequate to safeguard aquatic organisms.

Because it is not practical to generalize on the extent to which many of the important radioisotopes will be concentrated by aquatic organisms, nor on the extent to which they will be used for food by people, no attempt is made here to specify maximum permissible concentrations (MPC) for water in reference to uptake by the organisms. Rather, each case requires a separate evaluation that takes into account the peculiar features of the region. Such an evaluation should be approved by an agency of the State or Federal Government in each instance of radioactivity contamination in the environment. In each particular instance of proposed contamination, there must be a determination of the organisms present, the extent to which these organisms concentrate the ratioisotopes, and the extent to which man uses the organisms as food. The rates of release of radioisotopes must be based on this information.

Recommendation

Aquatic organisms concentrate radioisotopes to various degrees in their tissues. The concentration in sea water should be low enough so that the concentration in any aquatic species will not exceed Radiation Protection Guides of the U.S. Federal Radiation Council (1961)[401] for organisms harvested for use as human food. This recommendation is based upon the assumption that radiation levels which are acceptable as human food will not injure the aquatic organisms including wildlife.

SEWAGE AND NUTRIENTS

Magnitude of the Problem

The discharge of municipal sewage is a major factor affecting the water quality of receiving systems. Because the amount of municipal waste produced is directly related to the human population, the unit emission rates together with information on the number of people using a system provide an accurate estimate of the load that is imposed on a particular estuary or section of coastal water.

The effect of sewage discharges on water quality varies widely and depends on (1) its composition and content of toxic materials, (2) the type and degree of treatment prior to discharge, (3) the amount released, (4) the hydrodynamics of the receiving waters, and (5) the response of the ecosystem. Increasing human population and affluence have resulted in increasing amounts of domestic and industrial wastes. However, because the kind and degree of treatment often can be improved, it should be possible to cope with this pollution problem and to maintain or improve the quality of the marine environment.

In most cases the discharge of sewage effluent is intentional and the source of sewage and sewage treatment products entering marine ecosystems can be described more accurately than the sources of other pollutants entering the ecosystem. The volume of discharges and certain aspects of their composition, specifically, the amount of organic matter and the inorganic nutrients, can be monitored continuously by existing automated methods. Average values for some important constituents and their emission rates in a densely populated coastal area are given in Table IV-8.

TABLE IV-8—Average Sewage Emissions for a Densely Populated Area

Constituent	Mass emission rate (tons/day)[a]	Unit emission rate (lb/capita/day)
Dissolved solids	3,600	1.03
Suspended solids	565	0.162
BOD	560	0.160
Total nitrogen (N)	165	0.047
Phosphate (PO_4)	100	0.029

[a] For 700 mgd of sewage; population of 7 million.
NAS-NRC Committee on Oceanography 1970[417].

Runoff from agriculture areas is an important factor in the nutrient enrichment of freshwater systems, but it is less important to marine systems because relatively fewer farms are concentrated on estuaries and coasts. Nevertheless, agricultural practices should be considered. Pesticides, fertilizers and animal wastes may be carried by rivers into estuaries. Runoff from duck farms was involved in a study on excessive nutrient enrichment by Ryther (1954).[422] Commoner (1970)[404] has emphasized that in the United States during the last twenty-five years the amount of nitrogen used in agriculture has increased fourteenfold while the amount of nitrogen released via sewage has increased only seventy per cent.

In addition to degradable organic materials derived from fecal and food wastes, municipal sewage also contains a wide variety of "exotic" or synthetic materials that are nondegradable or degrade slowly and only under special conditions (e.g., petroleum residues, dissolved metals, detergents, dyes, solvents, and plasticizers). Some of these adversely affect the biota of receiving waters, and many interfere with the biological degradation of organic matter either in the treatment plant or in the environment. Because waste treatment technology currently in use is designed to treat the fecal and food materials derived from organic wastes, an operational definition of municipal sewage "exotics" is all those materials not derived from fecal or food sources. If the exotic materials accumulate in the receiving ecosystem, the capacity for recycling of the degradable organic materials may be reduced.

Oxygen Depletion

Efficient biological degradation of organic materials requires dissolved oxygen, and overload of sewage in receiving waters can result in oxygen depletion and secondary effects such as objectionable odors, plant and animal die-off, and generally decreased rates of biological degradation. Such effects can also be created by excessive algal growth and subsequent die-off.

The most widely used method for estimating the organic pollution load of a waste is the 5 day Biochemical Oxygen Demand Test (BOD_5). Discussions of the test (Fair et al. 1968,[407] Standard Methods 1971[408]) and its limitations (Wilhm and Dorris 1968[426]) are available. Among the important limitations of the BOD_5 are: it does not indicate the presence of organics which are not degraded under the prescribed conditions; it assumes that no toxic or inhibitory materials will affect microbial activity; and it does not measure the nitrogeneous oxygen demand of the organic waste. The chemical oxygen demand (COD) is an alternate procedure for determining the amount of oxidizable material in a water sample. However, it does not indicate the nature of biological oxygen consumption in a given time, and it does not distinguish between inorganically and organically oxidizable materials. Both BOD_5 and COD measurements must be recognized as being only partial descriptions of the sewage load of a receiving water. While BOD_5 and COD measurements are useful for evaluating treatment systems, these two measurements do not adequately assess the environmental impact of a given sewage load (Wilhm and Dorris 1968).[426]

Excessive Nutrient Enrichment

Marine plants, like those on land and in fresh water, require fertilizing elements essential for their growth and reproduction. These essential elements are natural constituents of municipal sewage and the amount that can be added to the marine environment without deleterious effect is determined by the stimulated growth of aquatic plants. Even if the major share of the organic material is removed from the sewage in treatment plants, the growth of normal marine plants can increase if the fertilizing elements present in sewage are added to the environment. Sewage treatment plants are designed to remove the organic material and the suspended solids and to decrease the bacterial population by disinfection. In most cases, this is done by processes that release or "mineralize" the plant nutrients which then stimulate the growth of algae in the receiving waters. In only a few cases have efforts been made to remove these fertilizers from the effluent to prevent or reduce the excessive growth of plants in the aquatic environment.

In the marine environment, growth of phytoplankton is commonly limited by the availability of essential nutrients, the most important of which are phosphorus and nitrogen in available forms. In some cases, shortages of silicate can inhibit the growth of the diatoms and encourage growth of other species. In certain limited areas, other elements such as iron and manganese have been reported as limiting growth of algae, and the presence or absence of other growth stimulating substances, such as vitamin B_{12}, can influence both the amount and the character of plant species capable of growing. It should be noted that in the marine environment, several elements essential for plant growth such as potassium, magnesium, and sulfur, are present in great excess.

Organic material produced by natural phytoplankton populations produces an oxygen demand when the material is consumed or decomposed. Oxygen is produced by the process of photosynthesis, but this production occurs only near the surface during daylight when the amount of light penetrating the water is adequate. Due to the sedimentation of dead organic particulate material, decomposition usually takes place in the deep waters where photosynthetically produced oxygen is not available.

The amount of organic material which can be produced by marine phytoplankton as a result of the addition of fertilizing elements is dependent upon the composition of the organic material. Redfield et al. (1963)[42] give the following ratios as characteristic of living populations in the sea and of the changes which occur in amounts of various elements left in water as a result of algal growth:

$$\begin{array}{rrrl} \Delta O: & \Delta C: & \Delta N: & \Delta P = \\ 276: & 106: & 16: & 1 \text{ by atoms or} \\ 138: & 40: & 7\tfrac{1}{4}: & 1 \text{ by weight} \end{array}$$

In addition to the readily available forms of phosphorus and nitrogen (dissolved orthophosphate, ammonia, nitrite, and nitrate), organic forms of phosphorus and nitrogen may be made available by bacterial decomposition. Some dissolved organic nitrogen compounds are also available for direct assimilation.

It should be emphasized that these ratios are not constant in the rigorous sense of the stoichiometric ratios in chemistry. The plant cells can both enjoy a "luxury" consumption of each element (Lund 1950)[414] or survive nutritional deficiencies (Ketchum 1939,[410] Ketchum et al. 1949[411]). In terms of the total production of organic material these variations are important only when concentrations of the elements are unusually low. It has been shown, for example, in New England coastal waters that nitrogen is almost completely removed from the sea water when there is still a considerable amount of phosphorus available in the system. Under these circumstances the plants will continue to assimilate phosphorus, even though total production of organic matter is limited by the nitrogen deficiency (Ketchum et al. 1958,[412] Ryther and Dunstan 1971[421]).

The amount of oxygen dissolved in sea water at equilibrium with the atmosphere is determined by salinity and temperature. Nutrient elements added to the marine environment should be limited so that oxygen content of the water is not decreased below the criteria given in the discussion of Dissolved Oxygen in this Section. In many polluted estuaries, the amount of fertilizing elements added in municipal sewage is sufficient to produce enough organic material to completely exhaust the oxygen supply during decomposition. The oxygen content of sea water and of fresh water at equilibrium with the atmosphere is presented for different temperatures in Table IV-9. For the purposes of this table, a sea water of 30 parts per thousand (‰) salinity has been used, which is characteristic of the nearshore coastal waters. The salinity effect on concentration of oxygen at saturation is minor compared to effects of temperature in the normal ranges found in coastal waters.

From the ratios of elements given above and the saturation values for oxygen, one can derive the effect of nutrient enrichment of marine waters. For example, from an addition of phosphorus and available nitrogen to final concentrations of 50 and 362.5 micrograms per liter respectively in the receiving water, enough organic material could be produced to remove 6.9 milligrams per liter of oxygen from the water. Data in Table IV-9 indicate that sea water with a salinity of 30 ‰ and a temperature of 25 C will contain, at saturation, 6.8 milligrams of oxygen per liter. This concentration of nutrients would thus permit the system to become anoxic and would violate the requirement that oxygen not be changed beyond levels expressed in the section on Dissolved Oxygen. Fresh water would contain 8.1 mg/l of oxygen at saturation at 25 C, so that the same amount of nutrient addition would remove 84 per cent of the available oxygen.

The example used might be considered to set an upper limit on the amount of these nutrients added to water. The actual situation is, of course, much more complicated. It is clear from the data in Table IV-9 that summer conditions place the most stringent restrictions on nutrient additions to the aquatic environment. Furthermore, the normal content of nutrients in the natural environment has to be considered. If these were already high, the amount of nutrients that could be added would have to be reduced. As mentioned above, the ratio of elements present in the natural environment would also be important. Nitrogen is frequently the element in minimum supply relative to the requirement of the phytoplankton, and addition of excess phosphorus under these circumstances has less influence than addition of nitrogen. Differences in the ratios of nitrogen to phosphorus may also modify the type of species present. Ryther (1954),[422] for example, found that unusually low nitrogen to phosphorus ratios in Moriches Bay and Great South Bay on Long Island, New York, encourage the growth of microscopic forms of *Nannochloris atomus* at the expense of the diatoms normally inhabiting this estuary.

Many forms of blue-green algae are capable of fixing nitrogen from the gaseous nitrogen dissolved in sea water. Nitrogen deficiencies could be replenished by this mechanism so that decrease in phosphorus content without concomitant decrease in nitrogen content might still lead to overenrichment, as well as shift the dominant phytoplankton population.

Oxygen content of upper water layers can be increased by exchanges with the atmosphere. This process is proportional to the partial pressure of oxygen in the two systems so that the more oxygen deficient the water becomes, the more rapid is the rate of replacement of oxygen in the water by atmospheric oxygen. Finally, mixing and dilution of the contaminated water with adjacent bodies of water could make additional oxygen available. All of these variables must be considered in order to determine acceptable levels at which nutrients present in sewage can be added to an aquatic environment. In fact, many polluted estuaries already contain excessive amounts of these fertilizing elements as a result of pollution by municipal sewage.

The effects of ratios of elements discussed above have a very important bearing upon some of the methods of control. For example, the removal of phosphates alone from the sewage will have an effect upon the processes of overenrichment only if phosphorus is indeed the element limiting production of organic matter. When nitrogen is limiting, as it is in New England coastal waters according to Ryther and Dunstan (1971),[423] the replacement of phosphorus by nitrogen compounds, such as nitrilotriacetate (NTA) could be more damaging to the ecosystem than continued use of phosphate-based detergents.

Pathogenic Microorganisms

The fecal coliform index is the most widely used microbiological index of sanitary quality of an estuary. Fecal coliform indices represent a compromise between the ideal of direct determination of bacterial and viral pathogens in time-consuming laboratory procedures, and the indirect, less indicative but practical exigencies. Laboratory methods for quantitative enumeration of virus currently are being developed and their present status is one of promise, but more time is needed for their evaluation. Bacterial pathogen detection frequently requires special laboratory attention.

Virus, in general, may exhibit considerably longer survival times in water and shellfish as compared to fecal coliform bacteria. Under these circumstances a negative *E. coli* test can give a false impression of the absence of viral pathogens (Slanetz et al. 1965,[424] Metcalf and Stiles 1968[illegible]). Fecal coliform multiplication may possibly occur in polluted waters leading to further difficulties in interpreting sanitary quality.

Disinfection of waste water by chlorine is effective in removing most pathogenic bacteria but unpredictable in re-

TABLE IV-9—Effects of Salinity and Temperature on the Oxygen Content of Water in Equilibrium with Air at Atmospheric Pressure

Temperature C	Salinity ‰	Oxygen mg/l	Salinity ‰	Oxygen mg/l
25	30	6.8	0	8.1
20	30	7.4	0	8.9
10	30	9.1	0	10.9
0	30	11.66	0	14.15

Richards and Corwin 1956[431].

ducing the number of viruses. Differences in resistance of bacteria and virus to chlorination may result in the appearance of infectious virus in treated effluents devoid of bacteria. Failure to demonstrate the presence of viruses would be the best way to insure their absence, but such capability awaits development of methods adequate for quantitative enumeration of virus in water.

The pollution of estuaries with waste products has led to the contamination of shellfish with human pathogenic bacteria and viruses. Outbreaks of infectious hepatitis and acute gastroenteritis derived from polluted shellfish have reinforced concern over the dangers to public health associated with the pollution of shellfish waters. The seriousness of viral hepatitis as a world problem has been documented by Mosley and Kendrick (1969).[416] Transmission of infectious hepatitis as a consequence of sewage-polluted estuaries has occurred through consumption of virus-containing shellfish, either raw or improperly cooked. Nine outbreaks of infectious hepatitis have been attributed to shellfish (Liu 1970).[413] Contamination of water by sewage leads to the closing of oyster beds to commercial harvesting, denying public use of a natural resource and causing economic repercussions in the shellfish industry. (See the discussion of Shellfish in Section I on Recreation and Aesthetics.)

Sludge Disposal into Marine Waters

Dumping of sewage sludge in the ocean continues and this practice, although at present indispensable, constitutes a loss of one resource and potential danger for another. A study on the New York Bight sludge and spoil dumping area has shown that an accumulation of toxic metals and petroleum materials appear to have reduced the abundance of the benthic invertebrates that normally rework the sediments in a healthy bottom community (Pearce 1969).[419]

Deep Sea Dumping

Biological degradation of organic waste materials is generally affected by micro-biota and chemophysical environmental factors. The deep sea is increasingly considered for the disposal of organic waste materials. A recent study (Jannasch et al. 1971)[410] has shown that rates of bacterial activity in degrading organic materials was slowed by about two orders of magnitude at depths of 5,000 to 15,000 feet as compared to samples kept at equal temperatures (38 F) in the laboratory. Since (a) the disposal of organic wastes should be designed on the basis of rapid decomposition and recycling, and (b) there is no control of the processes following deep-sea disposal, this environment cannot be considered a suitable or safe dumping site.

Potential Beneficial Uses of Sewage

Light loads of either organic-rich raw sewage or nutrient-rich biological treatment (secondary) effluent increase biological productivity. Except for short-term data on increased fish and shellfish production, beneficial effects have rarely been sufficiently documented, but at the present time several active research programs are underway. Some degree of nutrient enrichment exists today in most estuaries close to centers of populations. These estuaries remain relatively productive and useful for fishing and recreation. Certain levels of ecosystem modification via organic and nutrient enrichment appear to be compatible with current water uses; however, subtle changes in ecosystems may be accompanied by later, more extensive change.

The possibility of intensive use of essential plant nutrients in waste material to increase the harvestable productivity of estuarine coastal systems has been suggested as a logical way to treat sewage and simultaneously derive an economic benefit. Aquaculture systems would essentially be an extension of the waste treatment process. Conceptually, aquaculture is a form of advanced treatment. The limiting factor involves problems presented by toxic synthetic chemicals, petroleum, metals, and pathogenic microorganisms in effluents of conventional biological treatment plants.

Rationale for Establishing Recommendations

It is conceptually difficult to propose a level of nutrient enrichment that will not alter the natural flora because seasonal phytoplankton blooms with complex patterns of species succession are an integral part of the ecology of estuarine and coastal waters. The timing and intensity of blooms vary from year to year and patterns of species succession are frequently different in successive years. The highly productive and variable ecology of estuaries makes it difficult to differentiate between the early symptoms of artificial nutrient enrichment and natural cyclic phenomena. In addition, there have already been major quantitative and qualitative changes in the flora of marine waters close to centers of population. These changes are superimposed on the normal patterns of growth and may not in themselves impair the recreational and commercial use of waters.

Simulation modeling has been used to predict the total phytoplankton response to given nutrient inputs with success by O'Connor (1965)[418] and DiToro et al. (1971)[405] in the San Joaquin Estuary and by Dugdale and Whitledge (1970)[406] for an ocean outfall. Their models predict the phytoplankton response from the interaction of the kind and rate of nutrient loading and the hydrodynamic dispersal rates. This technique, although not perfect, facilitates evaluation of the ecological impact of given nutrient loads, but does not help in deciding what degree of artificial enrichment is safe or acceptable.

Recommendations

- **Untreated or treated municipal sewage discharges should be recognized as a major source of toxic substances. Recommendations for these constituents will limit the amount of sewage effluent that can be dispersed into estuaries. Reduced degradation rates of highly dispersed materials**

should be considered if the effluent contains refractory organic material. Undegradable synthetic organic compounds do not cause oxygen depletion but can still adversely affect the ecosystem. Maintenance of dissolved oxygen standards will not prevent the potentially harmful buildup of these materials. Specific quantitative analyses should be done to identify and assess the abundance of these compounds.

- **The addition of any organic waste to the marine environment should be carefully controlled to avoid decomposition which would reduce the oxygen content of the water below the levels specified in the recommendations for oxygen.**
- **Neither organic matter nor fertilizers should be added that will induce the production of organic matter by normal biota to an extent causing an increase in the size of any natural anoxic zone in the deeper waters of an estuary.**
- **The natural ratios of available nitrogen to total phosphorus should be evaluated under each condition, and the element actually limiting plant production should be determined. Control of the amount of the limiting element added to the water will generally control enrichment.**
- **If the maximum amounts of available nitrogen and phosphorus in domestic waste increase the concentration in receiving waters to levels of 50 micrograms per liter of phosphorus and 360 micrograms per liter of nitrogen, enough organic matter would be produced to exhaust the oxygen content of the water, at the warmest time of the year under conditions of poor circulation, to levels below those recommended (see p. 275). These concentrations of nutrients are clearly excessive.**
- **The potential presence of pathogenic bacteria and viruses must be considered in waters receiving untreated or treated municipal sewage effluents. The present quality standards for fecal coliform counts (see pp. 31–32) should be observed. The procedures for the examination of seawater and shellfish as recommended by Hosty et al. (1970)[408] should be used.**
- **Disposal of sludge into coastal waters may adversely affect aquatic organisms, especially the bottom fauna. Periodic examination samples should determine the spread of such an operation to aid in the control of local waste material loads. The probable transport by currents should be carefully considered. The dumping of sludge into marine waters should be recognized as a temporary practice.**
- **Disposal of organic wastes into the deep-sea is not recommended until further studies on their fate, their effect on the deep-sea fauna, and the controllability of such a procedure have been completed.**

SOLID WASTES, PARTICULATE MATTER, AND OCEAN DUMPING

Disposal of solid wastes has become one of the most urgent and difficult problems in crowded urban centers. Ocean disposal of these waste materials is receiving increased attention as land suitable for disposal becomes increasingly difficult to find.

Solid wastes are of many types and each may have a different impact on the marine environment. Household and commercial rubbish as well as automobiles and sewage sludge are disposed of at sea. Industrial wastes may be either solid or dissolved material, of varying toxicity. Harbor channels need continuous dredging, temporarily increasing the suspended sediment load, and the spoils often are dumped in coastal waters. Building rubble and stone also often are placed in the sea. The impact of disposal of these different materials into the ocean will range from innocuous to seriously damaging.

Particulate material is also discharged to the ocean by surface runoff, sewage outfalls, and storm sewers (Municipality of Metropolitan Seattle 1965).[464] Much of this material settles to the bottom at or near the discharge site (Gross 1970).[443] An increasingly important method of disposal is that of barging solids offshore to be dumped in coastal areas. Table IV-10 shows compilation of the amounts of wastes barged to sea in 1968 on the Pacific, Atlantic, and Gulf Coasts (Smith and Brown 1969).[476]

Dredge Spoils

Dredge spoils make up a major share of sea disposal operations. Their composition depends upon the source from which they were obtained. Saila et al. (1968)[472] were able to differentiate between dredged spoil from Providence Harbor dumped offshore and sediments of the natural bottom in the dumping area (Rhode Island Sound). Gross (1970)[443] suggests that dredge spoil generally consists of a mixture of sands, silts, and wastes which form the surface deposits in harbors. He compared minor element concen-

TABLE IV-10—Ocean Dumping: Types and Amounts, 1968

Waste type	Atlantic	Gulf	Pacific	Total
	(In tons)			
Dredge spoils	15,808,000	15,300,000	7,320,000	38,428,000
Industrial wastes	3,013,200	636,000	981,300	4,630,500
Sewage sludge	4,477,000	0	0	4,477,000
Construction and demolition debris	574,000	0	0	574,000
Solid waste	0	0	26,000	26,000
Explosives	15,200	0	0	15,200
Total	23,887,400	15,966,000	8,327,300	48,210,700

Council on Environmental Quality 1970[illegible].

trations in harbor sediments, dredged wastes, and continental shelf sediments. The median values of observed concentrations were clearly different, although the ranges of concentrations overlapped.

The proportion of dredging spoils from polluted areas is illustrated in Table IV-11.

A variety of coastal engineering projects involve changes in suspended loads and sedimentation (Ippen 1966,[447] Wicker 1965[illegible]). Because important biotic communities may inhabit the sites selected for these projects, conflicts arise concerning navigational, recreational, fisheries, conservation, and municipal uses of the areas (Cronin et al. 1969).[436] Although our knowledge about the effects is limited and the literature is widely scattered, Copeland and Dickens (1969)[433] have attempted to construct a picture of how dredging affects estuarine ecosystems from information gathered in the upper Chesapeake Bay, Maryland, Redfish Bay, Texas, and an intracoastal canal in South Carolina.

The biological effects of suspended loads, sedimentation, dredging methods and spoil disposal may range from gross damage, such as habitat destruction and smothering, to more subtle effects under low but chronic conditions of sedimentation over long periods of exposure. The channelization, dumping of spoils, dredging, and filling in the Gulf Coast estuaries had destroyed roughly 200,000 acres of swamp, marsh, and bay bottom areas by 1968 (Chapman 1968,[432] Marshall 1968[459]).

Mixtures of clays, silts, fine sands, and organic matter, sometimes referred to as "faunally rich muddy sand," tend to support larger benthic populations than coarse clean unstable sands, gravels, or soft muds (Carriker 1967)[430] over or through which locomotion may be difficult (Yonge 1953).[482] Close relationships exist between the presence of organic matter, the mechanical nature of sediments, and infaunal feeding habits (Sanders 1956,[473] 1958,[474] McNulty et al. 1962,[461] Brett cited by Carriker 1967[430]).

Ten years after dredging Boca Ciega Bay invertebrate recolonization of canal sediments (92 per cent silt and clay; 3.4 per cent carbon) was negligible. None of 49 fish species caught in these canals (as compared to 80 species in undredged areas) was demersal, apparently because of the lack of benthic fish food organisms on or in the canal deposits (Taylor and Saloman 1968).[477] Breuer (1962)[429] noted that layers of dead oyster shell in South Bay corresponded to layers of deposited spoil from dredging and redredging of the Brownsville Ship Channel. He thought that this suggested destruction of South Bay oyster populations with each dredging operation.

Pfitzenmeyer (1970)[470] and Flemer et al. (1967)[441] noted a 71 per cent reduction in average number of individuals and a marked reduction in diversity and biomass in a spoil area in upper Chesapeake Bay after dredging ceased. One and one half years after dredging, the number of individuals and species diversity of the spoil disposal area, but not in the channel, were the same as those of the surrounding area.

In lower Chesapeake Bay, Harrison et al. (1964)[444] observed a transitory effect of a dredging and spoil disposal operation on infauna. Resettlement of the dredged and disposal areas was very rapid by active migration and hydrodynamic distribution of juveniles.

Mock (1967)[463] noted that an unaltered shore in Clear Lake, Texas, produced 2.5 times more post larval and juvenile brown shrimp (*Penaeus aztecus*) and 14 times more post larval and juvenile white shrimp (*Penaeus setiferus*) than a similar bulkheaded shore. In a laboratory study using similar substrates, Williams' (1958)[481] data suggested that the type of substrate may exert its influence through its effect on available cover, although a contributing factor may be the different food content of the substrate.

Bayless (1968)[427] observed higher average hatches of striped bass eggs (*Morone saxatillis*) on coarse sand (58.9 per cent) and a plain plastic pan (60.3 per cent) than on silt-sand (21 per cent), silt-clay-sand (4 per cent) or muck detritus (none). These results tend to support Mansueti's (1962)[458] and Huet's (1965)[446] contention that deposition of suspended matter may interfere with or prevent fish reproduction by destruction of demersal eggs in upper estuarine areas.

TABLE IV-11—Estimated Polluted Dredge Spoils

	Total spoils (in tons)	Estimated percent of total polluted spoils*	Total polluted spoils (in tons)
Atlantic Coast	15,800,000	45	7,120,000
Gulf Coast	15,300,000	31	4,740,000
Pacific Coast	7,320,000	19	1,390,000
Total	38,420,000	34	13,250,000

* Estimates of polluted dredge spoils consider chlorine demand; BOD; COD; volatile solids; oil and grease; concentrations of phosphorous, nitrogen, and iron; silica content; and color and odor of the spoils.

Council on Environmental Quality 1970[434]

Sewage Sludges

Sewage sludges contain about 5 per cent solids which consist of about 55 per cent organic matter, 45 per cent aluminosilicates, and tend to contain concentrations of some heavy metals at least ten times those of natural sediments (Gross 1970).[443]

Sewage sludge has been dumped off New York Harbor since 1924 in the same area. Studies by Pearce (1970a,[465] b)[466] show that the normal bottom populations in an area of about 10 square miles have been eliminated and that the benthic community has been altered over an area of approximately 20 square miles. Even the nematodes, unusually tolerant to pollution, are relatively scarce in the smaller area. In areas adjacent to the sewage sludge disposal area the sea clams have been found to be contaminated by enteric bacteria and the harvest of these clams in this area has been prohibited. The oxygen content of the water near the bottom is very low, less than 10 per cent of saturation in August, the warmest time of year. Chemical analysis of the sludge deposits have shown not only high organic content but also high concentrations of heavy metals and petrochemicals. In this area of the New York Bight, fin-rot disease of fish has been observed and is being investigated (Pearce 1970b).[466] In laboratory tests it has been shown that sludge deposits can cause necrosis of lobster (*Homarus americanus*) and crab shells and tend to clog their gills so that survival of these species in contact with the sludge deposits is very brief. In other laboratory experiments, organisms given a choice of substrate tend to avoid the sludge material in favor of the walls of the container or other surfaces that were made available (Pearce 1970b).[466] These studies have indicated that the disposal of sewage sludge has had disastrous ecological effects on the populations living on or near the bottom.

Many aspects associated with sludge dumping in the New York Bight require further investigation. It is not known, for example, how much of the material being dumped there is accumulating and how much is being decomposed. The effects of heavy metals, of oxygen-demanding materials, and of other components are imperfectly understood. When the rate of delivery of organic waste materials to an aquatic environment exceeds its capacity to recover, the rate of deterioration can be rapid. If, or when, sewage sludge disposal in this particular area of the New York Bight is terminated studies could determine whether the bottom populations can repopulate the area.

Solid Wastes

The amount of household and commercial rubbish to be disposed of in the United States is about 5 lbs per capita per day and is expected to increase to 7-½ lbs per capita per day (for a larger population), by the end of the present decade. Proposals have been made to collect and bale waste for transportation to the sea where it would be dumped in waters 1000 meters deep or more. It would be necessary that the bales be compacted to a density greater than sea water so that they would sink, and that no loose floating objects would be released from the bale. Among the suggestions made is that the bales be wrapped in plastic to avoid any leaching from the contents.

Pearce (1971)[468] reports that bales of compacted garbage wrapped in plastic and reinforced paper disintegrated in a few weeks when placed in water 10 to 20 meters deep off the coast of New Jersey. Compacted bales of refuse were also anchored at a depth of 200 meters off the Virgin Islands Pearce (1970c).[467] These were retrieved and inspected after approximately three months of exposure. Little growth had occurred on the surface of the bales, but some polychaete worms had penetrated the bales to a depth of 2–3 cm., and the material within the bale had decomposed to a limited extent. Relatively high counts of total coliform bacteria (96,000 Most Probable Number, MPN) and of fecal coliforms (1,300 MPN) were found in materials retrieved from the interior of the bales, indicating prolonged survival or growth of these nonmarine forms and suggesting a possible hazard of introduction of pathogens to the sea. The ecological effects of disposing of these materials are inadequately known.

Disposal of solid wastes, including dredging spoils and sewage sludge into the deep waters off the edge of the Continental Shelf (more than 200 meters) has been frequently suggested as a way to protect the inshore biota. However, the rate of decomposition of organic material at the high pressure and low temperature of the deep sea is very much slower than it would be at the same low temperature at atmospheric pressure (Jannasch et al. 1971).[449] The organisms in the deep sea have evolved in an extremely constant environment. They are, therefore, unaccustomed to the unusual stresses which confront organisms in more variable situations typical of coastal waters. Biologists interested in studying the bottom populations of the deep sea are extremely concerned about altering these populations before there is an opportunity to study them thoroughly.

Industrial Wastes

A wide variety of industrial waste is being dumped at sea. If this is discharged as a solution or slurry from a moving ship or barge it will be diluted in the turbulent wake and by the normal turbulence of the sea (Ford and Ketchum 1952).[442] The recommendations for mixing zones (p. 231) and for the constituents of specific waste material included should be applied to each such operation.

One such operation which has been extensively studied is the disposal of acid-iron wastes in the New York Bight (Redfield and Walford 1951,[471] Ketchum et al. 1951,[451] Vacarro et al. 1972,[475] Wiebe et al. *in press* 1972[479]). Even though this disposal has proceeded for over twenty years, no adverse effects on the marine biota have been demonstrated. The acid is rapidly neutralized by sea water and the iron is precipitated as nontoxic ferric hydroxide. This is a flocculant precipitate and the only accumulation above normal background levels in the sediments appears to be in the upper end of the Hudson Canyon, close to the specified dumping area. The so-called "acid grounds" have become a favored area among local blue fishermen. More toxic materials would clearly present an entirely different set of problems. This illustrates the need for a rational approach to problems of ocean dumping.

Other Solid Wastes

Automobiles are sometimes dumped at sea, and some work has been done on an experimental basis in an effort to determine whether artificial reefs can be created from them to improve sport fishing. There is evidence that the number of fish caught over these artificial reefs is greater than over a flat level bottom, but it is not yet certain whether this represents an aggregation of fishes already in the area or an actual increase in productivity.

Disposal of building rubble (brick, stone, and mortar) at sea is not widely practiced. Presumably, this material could form artificial reefs and attract populations of fish, both as a feeding ground and by providing some species with cover. Obviously, the bottom organisms present would be crushed or buried, but Pearce (1970a,[465] b)[466] found no permanent detrimental effects in the building rubble disposal site off New York City.

Suspended Particulate Materials

In addition to specific waste disposal operations, suspended particulate material, seston, may be derived from other sources, and have a variety of biological effects. Particulate material can originate from detritus carried by rivers, atmospheric fallout, biological activity, chemical reactions, and resuspension from the bottom as a result of currents, storms, or dredging operations. The particles introduced by rivers can be rock, mineral fragments, and clay serving as a substrate for microorganisms or affecting light transmission in the water column. In addition, organic matter fragments, which make up 20 to 40 per cent of particles in coastal waters (Biggs 1970,[425] Manheim et al. 1970[457]) may comprise 50 per cent to 80 per cent of suspended material further offshore. Particle concentrations generally range from 1 to 30 mg/l in coastal waters to about 0.1 to 1 mg/l at the surface in the open ocean. Higher concentrations occur near the bottom.

The estimated yearly sediment load from rivers to the world oceans is estimated at 20 to 36 × 10^9 tons with 80 per cent originating in Asia (Holeman 1968).[445] Much of this load is trapped in estuaries and held inshore by the general landward direction of subsurface coastal currents (Meade 1969).[462] Gross (1970)[443] suggests that 90 per cent or more of particles originating from rivers or discharged to the oceans settles out at the discharge site or never leaves the coastal zone.

Average seston values may more than double from natural causes during a tidal cycle. Biggs (1970)[425] observed concentrations in the upper Chesapeake Bay ranging from less than 20 mg/l to greater than 100 mg/l during a single day. Resuspension of bottom sediments by storm waves and currents induced by wind were responsible for this range of concentrations. Masch and Espey (1967)[460] found that the total suspended material concentrations in Galveston Bay, Texas, ranged from 72 mg/l in the surface water of the ship channel to over 150 g/l six inches above the bay bottom near dredging operations. Normal background concentrations in Galveston Bay during times of strong wind action were 200 to 400 mg/l. Background values observed by Mackin (1961)[458] in Louisiana marshes ranged from 20 to 200 mg/l. Depending on the amount of overburden, operation times, and rate of discharges, Masch and Espey (1967)[460] recorded suspended fixed solids concentrations in dredge discharges ranging from 3,000 to 29,100 mg/l.

The basic relationships between physical and chemical aspects of suspended and deposited sediments and the responses of estuarine and marine organisms are poorly understood (Sherk 1971).[475] However, there is general agreement that particulate material in suspension or settling on the bottom can affect aquatic organisms both directly and indirectly, by mortality or decreased yield.

Particles suspended in the water column can decrease light penetration by absorption and scattering and thus limit primary productivity. Resuspended sediments exert an oxygen demand on the order of eight times that of the same material in bottom deposits (Isaac 1965).[445] Jitts (1959)[450] found that 80 to 90 per cent of phosphate in solution was absorbed by silt suspensions which might also modify the rate of primary production. However, exchange rates and capacity of sediment can maintain a favorable level of phosphate (1 micromole/l) for plant production (Pomeroy et al. 1965).[469] Carritt and Goodgal (1954)[431] postulated a mechanism for phosphate removal, transport, and regeneration by the sediment-phosphate sorption complex at different temperatures, pH values, and salinities.

Evidence tends to support the contention that nutrient fertilization and possible release of toxic materials can occur with resuspension of bottom material in the water column (Gross 1970).[443] This may occur during dredging, disposal and dumping operations, reagitation during storms or floods and from beach erosion. In upper Chesapeake Bay total phosphate and nitrogen were observed to increase over ambient levels by factors of 50 to 1,000 near an overboard spoil disposal project, but no gross effects were observed in samples incubated with water from the spoil effluent (Flemer et al. 1967,[441] Flemer 1970[440]).

Oyster and clam eggs and larvae demonstrate a remarkable ability to tolerate the variable turbidities of the estuarine environment at concentrations up to 4.0 g/l (Carriker 1967,[430] Davis and Hidu 1969[438]). Survival and growth of these egg and larval stages reported by Davis (1960)[437] and Loosanoff (1962),[452] however, indicated a significant effect on survival at suspended particle concentrations of as little as 125 mg/l. Earlier life stages of the oyster tend to be more sensitive to lower concentrations of suspended material than adults. However, the effects on survival and growth cannot wholly be attributed to particle sizes and concentrations since different particle types may have markedly different effects at similar concentrations. The adult American oyster (*Crassostrea virginica*) appears to be a remarkably silt-tolerant organism when not directly smothered by deposited sediments (Lunz 1938,[454] 1942[455]). Significantly, mortality of adult oysters was not evident with suspended sediment concentrations as high as 700 mg/l (Mackin 1961),[458] but there was a drastic reduction in pumping rates (57 per cent at 100 mg/l of silt) observed by Loosanoff and Tommers (1948)[453] and Loosanoff

(1962[452]). Apparently adult oysters may pump at reduced rates throughout most of their lives when the background suspended particulate matter persists at values observed by Biggs (1970)[428] and Masch and Espey (1967[460]).

Organisms that colonize hard surfaces must contend with a sediment mat of varying thickness. While motile fauna may be able to adjust to short range vertical bottom alterations from scour or deposition, "... the capacity and behavior of less motile estuarine benthos in adjustment to relatively rapid fluctuations in the bottom level are little known. Fixed epifauna, like oysters and barnacles, perish when covered by sediment, adjustment occurring only indirectly through later repopulation of the area from elsewhere" (Carriker 1967).[430]

The highly variable nature of suspended loads (Biggs 1970),[428] the resuspension of bottom accumulations by currents, tidal action and wind, and the feeding and filtering activities of benthic organisms complicate the determination of threshold values or limiting conditions for aquatic organisms. Data are difficult to compare because of differences in methods and approaches. This may indicate a lack of understanding of sedimentation and the difficulty in distinguishing between the effect of light attenuation by suspended particles and the effects of these particles on growth and physiology of estuarine and marine organisms (Municipality of Metropolitan Seattle 1965).[464] The observed responses of organisms may not be due to turbidity or total suspended sediment concentration, but to the number of particles, their densities, sizes, shapes, types, presence and types of organic matter and the sorptive properties of the particles.

Physical alterations in estuaries and offshore dumping have had obvious effects on estuarine and marine biological resources. These effects have been given little consideration in project planning, however, and little information exists concerning the magnitude of biological change because few adequate studies have been attempted (Sherk 1971).[475] Areas of high biological value, such as nursery grounds or habitats for commercially important species, must be protected from sediment damage (Municipality of Metropolitan Seattle 1965).[464] For example, the exceptionally high value of the Upper Chesapeake as a low salinity fish nursery area has been demonstrated (Dovel 1970).[439] Larvae and eggs are particularly sensitive to environmental conditions, and sediment-producing activities in this type of area should be restricted to seasons or periods of least probable effects.

Results reported from the study of this area, concerning seasonal patterns of biota, the nature of the sediments, and physical hydrography of the area, can be applied to the other areas being considered for dredging, disposal, and dumping. These data, in addition to careful pre-decision surveys or research conducted at the site under consideration should provide a guide to efforts to minimize damage and enhance desirable features of the system (Cronin 1970).[435]

Adequate knowledge of local conditions at sites selected for any sediment-producing activity is essential, however. This will generally require preproject surveys for each site selected because knowledge of ecological impacts of these activities is limited. Data should be obtained on the "... biological values of the areas involved, seasonal patterns of the biota, the nature of the sediments, physical hydrography of the area, and the precise location of productive or potential shellfish beds, fish nursery areas and other areas of exceptional importance to human uses..." which are close to or in the site selected (Cronin 1970).[435]

Appropriate laboratory experiments are also required. These should have value in predicting effects of sedimentation in advance of dredging operations. Eventually, the results of these experiments and field observations should yield sets of environmental conditions and criteria, for adequate coastal zone management and competent guidance to preproject decision making (Sherk 1971).[475]

The presence of major benthic resources (e.g., oyster beds, clam beds) in or near the selected area should be cause for establishment of a safety zone or distance limit between them and the sediment-producing activity. This would control mortality caused by excessive deposition of suspended particulate material on the beds and prevent spread of spoil onto the beds from the disposal or dumping sites. Biggs (1970)[428] found that the maximum slope of deposited spoil was 1:100 and the average slope was 1:500 in the Upper Chesapeake. These slopes may prove useful in estimating safety zone limits on relatively flat bottoms. At times, the safety zone would have to be quite large. For example, the areas in New York Bight which are devoid of naturally occurring benthos in the sewage sludge and dredging spoil disposal areas were attributed to toxins, low dissolved oxygen, and the spreading of the deposits (Pearce 1970a).[465] The presence or absence of bottom currents or density flows should be determined (Masch and Espey 1967).[460] If these are present, measures must be taken to prevent transport of deposits ashore or to areas of major benthic resources.

Tolerable suspended sediment levels or ranges should accommodate the most sensitive life stages of biologically important species. The present state of knowledge dictates that the critical organism must be selected for each site where environmental modification is proposed.

Recommendations

The disposal of waste materials at sea, or the transport of materials for the purpose of disposal at sea should be controlled. Such disposal should be permitted only when reasonable evidence is presented that the proposed disposal will not seriously damage the marine biota, interfere with fisheries operations or with other uses of the marine environment such as navigation and recreation, or

cause hazards to human health and welfare. The following guidelines are suggested:

- Disposal at sea of potentially hazardous materials such as highly radioactive material or agents of chemical or biological warfare should be avoided.
- Toxic wastes should not be discharged at sea in a way which would adversely affect the marine biota. The toxicity of such materials should be established by bioassay tests and the concentrations produced should conform to the conditions specified in the discussion of mixing zones (pp. 231–232).
- Disposal of materials containing settleable solids or substances that may precipitate out in quantities adversely affecting the biota should be avoided in estuarine or coastal waters.
- Solid waste disposal at sea should be avoided if floating material might accumulate in harbors or on the beaches or if such materials might accumulate on the bottom or in the water column in a manner that will deleteriously affect deep sea biota.

In connection with dredging operations or other physical modifications of harbors and estuaries which would increase the suspended sediment load, the following types of investigations should be undertaken:

- Evaluation of the range and types of particles to be resuspended and transported, where they will settle, and what substratum changes or modifications may be created by the proposed activities in both the dredged and the disposal areas.
- Determination of the biological activity of the water column, the sediment-water interface, and the substrate material to depths which contain burrowing organisms.
- Estimation of the potential release into the water column of sediments, those substances originally dissolved or complexed in the interstitial water of the sediments, and the beneficial or detrimental chemicals sorbed or otherwise associated with particles which may be released wholly or partially after resuspension.
- Establish the expected relationship between properties of the suspended load and the permanent resident species of the area and their ability to repopulate the area, and the transitory species which use the area only at certain seasons of the year.

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SOLID WASTES

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Section III
Freshwater Aquatic Life and Wildlife

(WQC 106-213)*

Introduction

The revision of the Report of the National Technical Advisory Committee on Water Quality Criteria (1968) attests to the rapid growth of knowledge of water quality requirements for aquatic life and wildlife. Where earlier criteria for protecting aquatic life relied extensively on laboratory studies of acute toxicity coupled with often arbitrary application factors, recent research into chronic, sublethal effects of pollutants has aided in estimating safe levels and deriving application factors with a firm basis. Research on chronic effects should be continued because knowledge in this area is far from complete.

Considerable data have been generated pertaining to the effects of specific pollutants on species that are of direct importance in the food chain of desirable species. The highest priority should now be given to research on the effects of pollutants on ecosystems because it is not possible to protect significant species unless their interdependence with other components of their environment, both living and nonliving, is recognized.

Much of the discussion in Section IV (Marine Aquatic Life and Wildlife) in the categories of Ecosystem Analysis; Communities, Populations, and Organisms; Nonliving Components of the Marine Ecosystem; and Assimilative Capacity of Marine Systems also apply to freshwater systems. Thus the recommendations made in that section should be considered in both contexts.

Interactions and bioaccumulation are common to several categories of research needs discussed here, and repeated mention of them throughout this report is intended to suggest that they be given high priority. Materials present at innocuous levels in water may reach dangerous concentrations in sediments due to the action of physical and chemical processes. Virtually

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*Page numbers indicated in parentheses throughout refer to pertinent discussions in the parent document, *Water Quality Criteria 1972*, which is available from the Superintendant of Documents, U.S. Government Printing Office, Washington, D.C.

(Extracted from: Research Needs in Water
Quality Criteria - 1972)

unexplored is the effect of microbial activity on pollutants. The discovery of bacteriological methylation of mercury demonstrated the significance of bacteria in altering the characteristics of toxic materials in an aquatic environment.

Studies of the accumulation of toxic compounds by organisms along with studies of the biological magnification of these residues are needed as bases for specification of acceptable levels of many toxicants. Such studies by their very nature would have to be conducted in the context of ecosystems.

Of somewhat lower priority is the need to develop new research methodologies and improve old ones. However, in the case of high-priority research in which existing methodologies are inadequate, the development of techniques assumes a greater importance.

Community Structure and Ecosystem Analysis

(WQC 109-110)

Research is urgently needed to obtain a better understanding of the structure and function of aquatic ecosystems. Organism interrelationships such as predator-prey, parasite-host, producer-consumer, and functions such as the production of metabolites essential to other organisms are so complex and so closely interrelated that the elimination of one species or a fraction of a population may affect the total community. Protecting the most sensitive species in an ecosystem may protect the entire ecosystem, but which species is the most sensitive is not always clear.

- *Consequently, it is necessary to undertake integrated studies of freshwater ecosystems in order to understand and ultimately to predict the response of an ecosystem to various pollutants and to physical manipulation of the environment.*

- *Previous efforts to this end have been severely handicapped by the inadequacy of existing methodology for ecosystem analysis. Development of biological scale models that can be manipulated in the laboratory should provide a research tool that is convenient as well as productive. The deliberate and controlled addition of pollutants to small, unpolluted bodies of water has been used to great advantage in the past in determining the broad ecological impact of pollutants. The establishment of research facilities such as the Experimental Lakes Area of Ontario (Johnson and Vallentyne, 1971) to continue such studies is indicative of the utility of this method of investigation.*

Assimilative Capacity
(WQC 111)

Some aquactic ecosystems have the capacity to assimilate wastes generated by human activities; however, in too many cases this assimilative capacity has been exceeded and ecosystems have been degraded. Better knowledge of the assimilative capacity of these systems would have facilitated their preservation and such knowledge is needed for the wise use of other ecosystems.

- *Research is needed to establish methodologies and guidelines for determining the assimilative capacities of various kinds of receiving systems. Flushing rates and physical, chemical, and biological interactions should be included. Attention should also be given to devising a systematic classification of freshwater bodies to facilitate application of the results of this research.*

Mixing Zones
(WQC 112-115)

A mixing zone is a region in which an effluent discharage is progressively diluted in the receiving system, and it has the potential for exposing aquatic organisms to unusually high concentrations of pollutants, although normally for only a short time. Because of these high concentrations, mixing zones are of special concern in establishing water quality criteria, and the following research needs are indicated:

- *The time exposure of organisms to gradient concentrations of pollutants is of prime importance in establishing the size of mixing zones in flowing and standing waters. Although it is not particularly difficult to conduct laboratory experiments to determine the effects of short-term exposure to various dilutions of a given wastewater, it is much more difficult to predict the speed and direction of travel of motile organisms through a mixing zone. Studies of the behavior of organisms in and around mixing zones are of high priority if the dimensions of mixing zones are to be based on time exposure.*

- *Studies should also be undertaken on the effects of discharges that attract organisms. Problems of similar importance are the effect of overlapping mixing zones, especially when synergistic interaction of pollutants is possible; the relative effects of various discharage schemes (shoreline versus midchannel discharge, jet versus slow mixing); and determination of the necessary size of zones of passage.*

Biological Monitoring

(WQC 116-117)

The advent of biological monitoring of water quality, both in field surveys and in the management of waste streams, offers advantages of sensitivity and speed over many chemical analyses. Furthermore, aquatic organisms integrate all the stresses placed on an aquatic system and reflect the combined effect.

- *The first concern of future efforts in biological monitoring should be development of standardized procedures having application to a diversity of aquatic ecosystems. Such studies could most profitably be conducted along with chemical analyses of water with the ultimate objective of identifying useful predictors of potentially toxic conditions.*
- *Research should be initiated to determine those species best suited for monitoring various kinds of pollutants.*

Bioassays

(WQC 118-123)

Bioassays have been and will continue to be the basis for much of the knowledge on toxicity of many compounds. Much of the past work has employed acute-toxicity bioassays, but the former endpoint - death - has been supplanted by more subtle indicators of the well-being of an organism, such as reproductive success, rates of respiration and growth, and aspects of behavior. Long-term bioassays using some of these indicators often involve entire life cycles and several generations of test organisms.

In addition to their value as a tool for basic research, bioassays are essential in developing criteria for pollutants in specific bodies of water. Although bioassays for basic research might, to advantage, utilize selected strains of organisms and synthetic test media, tests aimed at specific applications should use organisms of local significance and water from the receiving systems of interest. In either case, the investigator should measure or control all water quality parameters, such as temperature, pH, hardness, and dissolved solids, that might affect the results either directly or by interacting with the toxicant under consideration.

- *With such extensive use of bioassays there has developed the need for standardized procedures and methods of reporting, particularly with regard to determining chronic and thermal effects and interactions of mixtures of toxic compounds. Also yet to be resolved are questions of the comparability of results of bioassays involving different organisms, and the applicability of laboratory data to the natural environment. Despite the progress that has been made, bioassays have not yet been conducted for a number of important* *compounds, as indicated in the sections on oils and toxic substances in this report.*

Physical Manipulation of the Environment
(WQC 124-125)

Human activities have resulted in numerous alterations of aquatic environments, some of which have had profound changes on ecosystems. Examples of these manipulations are channelization, dredging, filling, shoreline modification, clearing of vegetation, diking, leveling, sand and gravel removal, impounding, interbasin transfer, and covering land areas with impervious structures such as roads and buildings. Considerably more work is needed on the effects of these and other physical manipulations that affect rivers, lakes, estuaries, and coastal waters.

- *There is a critical need to evaluate fully the role of wetlands from the standpoint of their importance in nutrient retention and removal, and as nursery areas for various animals. There is a need to investigate the consequences of eliminating food-producing riffle areas and shelter areas along the banks of streams by channelization.*

Suspended Solids
(WQC 126-129)

Closely related to problems arising from physical manipulation of the environment are the effects of suspended solids. Clearing land of its vegetation for the construction of roads and buildings substantially increases the sediment yield from land areas. Similarly, alterations designed to enhance drainage by increasing the velocity of flow also increase the capacity of a stream to carry suspended matter. Although excessive amounts of suspended matter are known to be directly harmful to aquatic life because of abrasive action, clogging of gills, and visual impairment (and quantitative data are still needed in these areas), the greatest need is for research on the interactions of suspended matter with toxic substances.

- *The adsorption of metals, pesticides, and other toxicants onto suspended particles that subsequently settle should be investigated, since this provides a mechanism for concentrating toxic substances in bottom sediments of lakes and estuaries to the extent that benthic organisms can be threatened. In addition, the potential for release of these absorbed materials into the water column through biochemical reduction and resuspension of sediments by the action of vessels, floods, wind, and waves should be determined.*

Color

(WQC 130)

The color of water has not been considered a major cause for concern in aquatic ecosystems, but it is known that the presence of colored compounds can reduce photosynthesis. Whether color itself has any other effects is apparently not known; therefore, research should be undertaken to determine whether the color of water can have behavioral or physiological effects on aquatic life such as alteration of feeding and migration patterns or inhibition of photosynthesis to such an extent that symptoms of eutrophication might be masked.

Dissolved Oxygen

(WQC 131-135)

Dissolved oxygen concentration is one of the most important and most frequently monitored water quality characteristics. Because dissolved oxygen concentration is influenced by water temperature and concentrations of degradable organic matter, a knowledge of the dissolved oxygen requirements of aquatic organisms is essential to establishing criteria for oxygen-demanding pollutants and waste heat.

- *Despite the extensive literature on dissolved oxygen requirements of fish, there is little information on the requirements of most invertebrates. Additional information is needed on oxygen requirements of warm water fish from various regions. Of special interest are the dissolved oxygen minima tolerated by fish under a variety of stresses such as heat, toxicants, and extremes in pH. Also there is insufficient information on the effects of repeated exposure to extremes in dissolved oxygen concentration.*

Total Dissolved Gases

(WQC 135-139)

The total dissolved gas concentration in water is important because of the stress that supersaturation imposes on aquatic organisms. Bubbles released from supersaturated solutions on the body surface of small organisms can block the flow of water over respiratory surfaces and cause a change in buoyancy that could lead to predation or exposure to unfavorable water quality. Formation of bubbles within the tissues of fish (gas bubble disease) can cause severe damage or death.

The cause of these phenomena has been shown to be an excess of total dissolved gases and not just dissolved nitrogen. The following research is needed to provide a good data base for criteria on gas supersaturation:

- *Studies of the susceptibility of invertebrates and various species of fish to gas bubble disease are particularly necessary to assess the magnitude and importance of the problem. Once these have been established, a logical second step would be determination of chronic effects of gas supersaturation and its interaction with other stresses. Although several methods are available, there is a need for a standardized method of measuring the degree of gas saturation.*

pH

(WQC 140-141)

The pH of a solution is perhaps the most important variable in determining the chemical state of many constituents, such as ammonia, hypochlorite, cyanides, and sulfides, which appear to be most toxic in their unionized form. The toxicity of metallocyanide complexes is also affected by pH.

- *Research efforts should be directed toward determining the effect of pH on the toxicity of a wide variety of chemicals, particularly heavy metals.*

Dissolved Solids and Hardness

(WQC 142-143)

Although the concentration of total dissolved solids in the freshwater systems of the United States has not become so great that it constitutes a major threat to aquatic life, several points should be investigated.

- *On the basis of circumstantial evidence, Provasoli (1969) suggested that monovalent ions, particularly sodium, might be involved in promoting nuisance blooms of blue-green algae. The possible ramifications of this hypothesis justify additional research.*
- *Judd (1969) has shown that salt used for deicing resulted in profound changes in a small lake. The abundance of benthic organisms was affected, and vertical distribution of nutrients was altered as a result of salt-related disturbance of normal circulation and stratification. Studies of this kind should be extended to include the effect of fluctuations in salt concentration on stream biota. Such fluctuations could be harmful by themselves or by interaction with toxicants already present.*

Oils
(WQC 144-146)

Problems of oil pollution have received considerably more attention in the marine environment than in the freshwater environment; however, the consequences of a large oil spill in a confined area such as a river or lake would be far more serious than those of a spill in open waters. The likelihood of such a disaster is increasing because of the increased use of oil as a substitute for coal. Continuous or intermittent release of lesser quantities of petroleum products to inland waters as a result of refining, transportation, and use can be equally important and merits special attention in freshwater ecosystems.

- *The presence of floating oils in visible amounts is generally considered unsightly as well as harmful to aquatic life and wildlife; nonetheless, invisible surface films of oil should be examined closely for adverse biological effects attributable to adsorption onto the body surface of organisms, or to interference with the exchanges that normally occur at the air-water interface. The toxicity of oil dispersants and of various crude and manufactured oils also requires further investigation.*
- *Sedimented oils pose a problem that demands more immediate attention in both freshwater and the marine environments because of their persistence and widespread occurrence. Safe levels of sedimented oils have yet to be determined, and the implications of their ability to concentrate other toxic substances is unknown.*

Tainting Substances
(WQC 147-150)

Numerous chemical compounds have been found to impart such objectionable tastes, odors, and colors to edible aquatic species as to render them unfit for human consumption. Of these compounds, those that are of biological origin are particularly troublesome. Additional research should make it possible to establish criteria designed to limit the growth of the nuisance organisms involved.

- *This research would involve, first, a determination of the identity and numbers of nuisance algae and fungi associated with tainting the flesh of edible species, and second, studies of their physical and chemical requirements.*

Heat and Temperature

(WQC 151-171)

The rising demand on the nation's waters as a temporary sink for waste heat could constitute a threat to aquatic life. More information is needed on the effects on aquatic life of both prolonged exposure to elevated temperatures and short-term exposure to temperature extremes. For the research needs identified below, field studies may be preferred over laboratory studies. In past studies conducted near electric power plants, it has often been difficult to distinguish between thermal effects, physical damage at water intakes, and toxicity of biocides used to control fouling. Because of these difficulties, field study sites should be carefully designed.

- *Prolonged exposure to constant elevations above natural temperatures should be examined for possible adverse and beneficial effects on migration, behavior, reproduction, growth, and emergence of beneficial species as well as nuisance organisms. The effects on aquatic life and wildlife of thermal discharges that limit ice formation should be determined.*
- *Fluctuating temperatures are apparently of far more importance than the dominant temperature to the survival, growth, and reproduction of aquatic life. For several species of fish and for most invertebrates, we have no data on survival after brief exposure to both high and low temperature extremes. More research is needed to determine the rates at which aquatic organisms acclimate to temperature change, and to determine the chronic effects of continuously fluctuating water temperatures on aquatic organisms.*

Toxic Substances

(WQC 172-193)

Determination of safe concentrations of toxic substances has been the subject of extensive investigations and requires continued effort because of the large and increasing number of compounds involved, and because of the kinds of information needed for each compound. In theory, it should be necessary only to determine the effect of a given toxicant on the most sensitive life stage of the most sensitive organism in a given ecosystem; however, the identification of the species most sensitive to a particular toxicant does not usually become clear until after considerable damage has been done to the ecosystem. Even where damage is evident, it is often difficult to determine the causative agent because of the variety of stresses that might be encountered. Consequently, toxicity research generally involves the study of several species. Attention must be given to acute and chronic, lethal and sublethal effects as well as to residues of toxic compounds that are transferred through the food chain.

In addition to studies of the biological effects of toxicants, research is urgently needed on the interactions of toxicants with other components of the environment, both living and nonliving. Of particular concern is the response of aquatic life to exposure of combinations of toxicants where synergistic and antagonistic effects are possible.

Organic Mercury

Organic mercury in fresh water is of concern because of its high toxicity and potential for bioaccumulation and adverse effects on aquatic life. The discovery of microbial methylation of inorganic mercury has increased our awareness of the complexity of the mercury problem and made clear the general need for better understanding of the role of microorganisms in the aquatic environment.

- *Despite a growing body of data on residues of mercury found in aquatic organisms, much remains to be known about what tissue concentrations can be considered safe for aquatic organisms and their predators, and about the effects of long-term exposure to mercury on growth and reproduction.*
- *Efforts to determine the historical concentration of mercury from examination of museum specimens, sediment cores, and glacial ice should be continued so that addition of mercury to the environment in recent times can be fully evaluated.*

Phthalate Esters, Polychlorinated Biphenyls, and Related Compounds

Among the synthetic organic compounds found in the nation's freshwater systems, phthalate esters and polychlorinated biphenyls merit special attention because of their widespread use and, in the case of polychlorinated biphenyls, extreme toxicity. Efforts to assess environmental hazards associated with these compounds are of rather recent origin, and several kinds of fundamental information are lacking.

- *The acute and chronic toxicity of these compounds has been determined for only a few species of aquatic life and wildlife. Little is known about their potential for biological accumulation and magnification, or about the toxicity of possible contaminants in commercial formulations. Improved methods of analysis of environmental samples would be extremely helpful in both field and laboratory investigations of these compounds.*

Metals

The extensive literature on effects of metals on aquatic life in most cases permits estimation of safe levels or application factors for protecting aquatic organisms from metal poisoning. However, mechanisms by which metals may be concentrated in the environment are poorly understood, and the relative toxicities of the various forms of metals encountered are largely unknown.

- *The need for research on the adsorption of metals by suspended matter has been discussed. More information is required on the effects of natural organic chelating agents, pH fluctuation, and temperature on the toxicity of heavy metals, and on the role of the biota in transforming heavy metals once they enter the environment. Studies of the biological accumulation and magnification of metals in individuals and in food chains are also needed, particularly for those metals having radionuclides.*

Pesticides

The widespread and growing use of pesticides for controlling undesirable plant and animal life and their high toxicity to aquatic life demand broad knowledge of their effects.

- *For many of these compounds, such as those in Table 2, it is not possible to estimate safe levels on the basis of present information. The necessary studies of acute and chronic toxicity and potential for accumulation in food chains should be initiated, especially for the more widely used compounds.*
- *For those pesticides that have been studied, information is generally scanty on the effects of metabolites and decomposition products, and on the effects of pesticides on species already exposed to other stresses such as heat, toxicants, or oxygen deficiency.*

Other Toxicants

The report, *Water Quality Criteria, 1972* (National Academy of Sciences-National Academy of Engineering, *in press*) indicates a need for additional research into the effects of chlorine and phenolics in order to provide better estimates of concentrations that can be considered safe for aquatic life. More information is needed on the reactions of chlorine with other compounds in the environment, particularly organic compounds. The long-term effects of repeated exposure to chlorine and alternative disinfectants are largely unknown.

There is a wide variation in the concentrations of phenolics that have been found toxic to fish. This variation may be due to the large number of phenolic compounds that enter the environment, and to the fact that typical analytical procedures fail to distinguish among them. Some of the resulting confusion could perhaps be resolved in studies of the effects of specific phenolic compounds on aquatic organisms.

TABLE 2. Pesticides for Which Safe Levels Are Unknown

Common or trade name	Chemical name
Organophosphate Insecticides	
Abate	**0,0,0',0'-tetramethyl 0,0'-thiodi-*p*-phenylene phosphorothioate**
Azinphosethyl	**0,0-diethyl S-(4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl phosphorodithioate**
Carbophenothion	**S-((*p*-chlorophenyl) thio methyl) 0,0-diethyl phosphorodithioate**
Chlorothion	**0,0-dimethyl 0-(3-chloro-4-nitrophenyl) phosphorothioate**
Demeton	**0,0-diethyl 0(and S)-(2-(ethylthio) ethyl) phosphorothioates**
Methyl parathion	**0,0-dimethyl 0-*p*-nitrophenyl phosphorothioate**
Phorate	**0,0-diethyl S-(ethylthio)-methyl phosphorodithioate**
Ronnel	**0,0-dimethyl 0-2,4,5-trichlorophenylphosphorothioate**
Carbamate Insecticides	
Aminocarb	**4-dimethylamino-*m*-tolyl methycarbamate**
Bayer 37344	**4-(methylthio)3,5 xylyl methylcarbamate**
Baygon	**0-isopropoxyphenyl-methylcarbamate**
Herbicides, Fungicides and Defoliants	
Acrolein	**2-propenal**
Balan	**N-butyl-N-ethyl-α,α,α-trifluoro-2,6-dinitro-*p*-toluidine**
Bensulide	**0,0-diisopropyl-S-(2-benzenesulphonamido)-ethyl-dithiophosphate**
Chloroxuron	**N'-4-(4-chlorophenoxy) phenyl - N, N-dimethylurea**
CIPC (Chlorpropham)	**isopropyl *m*-chlorocarbanilate**

TABLE 2. Pesticides for Which Safe Levels Are Unknown–continued

Common or trade name	Chemical name
Dacthal (DCPA)	dimethyl tetrachloroterephthalate
DEF	S,S,S-tributyl phosphorotrithioate
Dexon	*p*-dimethylaminobenzenediazo sodium sulfonate
Difolatan	*cis*-N-((1,1,2,2-tetrachloroethyl)thio)-4-cyclohexene-1,2-dicarboximide
Dinitrobutyl phenol (Dinoseb)	2,4-dinitro-6-*sec*-butylphenol
Diphenamid	N,N-dimethyl-2,2-diphenylacetamide
2,4-D (PGBE)	propylene glycol butyl ether ester of 2,4-dichlorophenoxyacetic acid
2,4-D (IOE)	isooctyl ester of 2,4-dichlorophenoxyacetic acid
2,4-D (Diethylamine salts)	diethylamine salts of 2,4-dichlorophenoxyacetic acid
Endothall (disodium salt)	disodium salt of 7-oxabicyclo [2.2.1] heptane-2,3-dicarboxylic acid
Endothall (dipotassium salt)	dipotassium salt of 7-oxabicyclo [2.2.1] heptane-2,3-dicarboxylic acid
Eptam	S-ethyl dipropylthiocarbamate
Hyamine-1622	para di-isobutyl phenoxy ethocy dimethyl benzyl ammonium chloride (98.8%)
Hyamine-2389	methyl dodecyl benzyl trimethyl ammonium chloride (40%) and methyl doecyl xylylene *bis* (trimethyl ammonium chloride) (10%)
Hydrothal-47	di(N,N-dimethylalkylamine) salt of endothall (66.7%)
Hydrothal-191	mono (N,N-dimethylalkylamine) salt of endothall (53%)
Hydrothal-plus	dipotassium salt of endothall (22.1%) plus potassium salt of α-(2,4,5-trichlorophenoxy) propionic acid (25.3%)
IPC	isopropyl-N-phenylcarbamate
MCPA	2-methyl-4-chlorophenoxyacetic acid
Molinate	S-ethyl-hexahydro-1 H-azepine-1-carbothioate
Monuron	N'-(4-chlorophenyl)-N,N-dimethylurea
Paraquat	1,1'-dimethyl-4,4'-dipyridinium dichloride
Pebulate	S-propyl butylethylthiocarbamate

TABLE 2. Pesticides for Which Safe Levels Are Unknown – continued

Common or trade name	Chemical name
Picloram	4-amino-3,5,6-trichloropicolinic acid
Propanil	N-(3,4-dichlorophenyl) propionamide
Silvex (IOE)	isooctyl ester of α-(2,4,5-trichlorophenoxy) propionic acid
Silvex (potassium salt)	potassium salt of α-(2,4,5-trichlorophenoxy) propionic acid
Trifluaralin	α,α,α-triflouro-2,6-dinitro-N,N-dipropyl-*p*-toluidine
Vernolate	S-propyl dipropylthiocarbamate

Wildlife

(WQC 194-198)

Water quality criteria for protecting wildlife species are directed principally toward ensuring an adequate and safe food supply and an environment free of harmful substances such as oils and toxic chemicals. Fish-eating birds and mammals at the apex of food chains are especially vulnerable to toxicants exhibiting trophic accumulation; thus, a pressing research need in this area is to determine what residues of toxicants in foods derived from the aquatic environment are safe for wildlife, and to determine the corresponding concentration of these materials in water. Of particular concern in this regard are mercury and toxic organics such as polychlorinated biphenyls and pesticides.

A large number of waterfowl are poisoned annually by the ingestion of lead shot. Research should be initiated to determine whether this loss is of consequence to the long-term survival of waterfowl populations.

CASE PREPARATION AND COURTROOM PROCEDURE

I TYPES OF PROCEEDINGS IN WHICH WATER QUALITY EVIDENCE MAY BE USED

A Administrative Proceedings

1 Rule making

a Setting up of regulations having general application, e.g., stream classifications and implementation plan target dates

b Factors of safety and absolute prohibitions may be appropriate

2 Adjudications

a Determinations by agency having expertise with respect to particular discharge or discharger, e.g., approval of plans and specs and time schedule of a particular discharger

B Court Actions

1 Civil in behalf of state or federal government

a Actions to compel action or suspension of action - nuisance, health hazard, etc., --including court action following federal conference --hearing procedure

b Violations of Water Quality Standards

c Violations of Effluent Standards or discharge permits

d Tort or contract actions relating to design and/or operation of treatment facilities

2 Criminal (dependent on content of applicable statutes)

a Discharge of specific materials

b Discharges from specific industries

c Littering

d Discharges harmful to fish and/or crustaceans

e Discharges harmful to specific types of receiving waters

f Discharges of poisons

NOTE--In some of these situations doing the act may constitute the violation; in others proof of intent or knowledge of effects may also have to be proved.

3 Private actions for damages or to compel action

a Alleged harm to plaintiff, e.g., pollution of stream killing animals

C Procedural Matters

1 See Attached sheet "Administrative and Court Proceedings" on Burden of proof, fact finding, and methods of presentation of evidence.

D Classes of Evidence - General Rules

1 Facts - direct

a The material was floating from the outfall.

2 Derived values - expert testimony - test results and/or opinion as to effects

a The D.O. was zero; the waterway was polluted; the plant can be built in 6 months.

3 Hearsay

a Joe told me

4 Relevancy

5 Admissibility vs. weight

   a Even if admissible, the weight to be given is up to fact finder--credibility.

E Admissibility of Results of Sampling and Testing (Numbers)

   1 Sampling

      a Chain of custody

      b Tags, etc.

      c Containers

      d Place and time

      e Retention of samples (Proving that the sample represents what is at issue in the action (relevancy), that there has been no opportunity for tampering; and availability of portions for analysis by other side (non-transitory criteria)).

   2 Analysis

      a Who performed (Can identity of each participant be shown?)

      b Admission through supervisor - custodian

      c Scientific acceptance of method. Is there a particular method required to be used by the agency?

      d Propriety of conduct

      e Retention of bench cards and other indicia of results. (Your attorney can make arrangements to substitute copies for originals).

   3 Tests

      a Comparison with actual conditions

      b Mathematical models - how can a computer be cross-examined?

F Admissibility of Expert Opinion on Causes and Effects

   1 Who has special knowledge - and of what particular areas?

   2 Indicators

   3 Significance of numerical determinations or observations

   4 Consistency with own prior publications and testimony

   5 Have underlying facts been or need to be proved--first hand information of this and/or comparable situations.

   6 Use of treatises

G Conduct on the Witness Stand

   1 General

      a On direct - know what counsel will ask and let him know generally what you will answer, but don't make it sound rehearsed.

      b Use layman's language to extent possible.

      c Listen to question and answer it to best of your ability.

      d Speak so that court reporter, judge, jury, and counsel can hear you.

      e Speak in language that will be understood; don't talk down.

      f Answer only what you are asked --don't volunteer; however, answer with precision.

      g There is nothing wrong with asking to have a question repeated or rephrased.

      h There is nothing wrong with saying that you consulted with your attorney before you testified, but beware of the question "Did Mr. X tell you what to say?"

i There is nothing wrong with thinking out your answer before responding.

j You are not expected to know all the answers--if you do not know, admit it.

k Don't attempt to answer questions outside your area of personal knowledge (hearsay) or beyond your expertise. (Your may be an expert on conducting laboratory tests, but not on epidimeological inferences from results).

l Don't try to answer before the judge rules on objection.

m Show that you are an impartial dispenser of information and/or opinion, not a protagonist.

n Don't be afraid to admit what may appear to be damaging.

2 If you are testifying as an expert:

a Establish qualifications -- give information relevant to your area of expertise -- educational (including this course?), work, publications, number of times you have testified previously.

b Differentiate between physical facts (measurements and observations) and opinion (derived values).

c Be prepared to discuss theory (including assumptions) instruments used, techniques(including choice of a particular technique), physical limitations and errors, interferences.

d If experiments were conducted, be able to justify both as to theory and relevancy to this litigation.

e If you're being paid to testify, admit it.

3 Scientific personnel as advisers to counsel:

a Review and refamiliarize self with materials before you discuss with your attorney.

b Be in a position to present all facts known to you simply and concisely: Who, What, When, Where, and Why, How.

c Don't overlook facts and/or test results because you don't think they're important. Let attorney decide what he needs.

d Use of standard report forms

e Ability to recommend additional witnesses with needed specialized knowledge

f Ability to aid in cross-examination of other side's experts and reconcile opinions and/or results

g Be candid - sometimes better not to start a lawsuit or accept a settlement than lose in the end.

H Non-Verbal Presentation of Evidence

1 Exhibits - including photographs

2 Summaries

3 Business and/or government records

a Prepared contemporaneously and in usual course of activities

4 Pre-prepared direct examination

a Usually limited to actions before ICC, FPC, and other federal agencies.

I Criminal Procedure

1 Privilege Against Self Incrimination (available only to persons)

a Warning and suspects

b Effect of duty to report spills

  c Effect of duty to obtain license or permit and/or furnish operating reports

  d Immunity from prosecution

2 Double Jeopardy

3 Unreasonable search and seizure

  a Available to persons and corporations

4 Procedures and need for arrest and search warrants --possible cause

This outline was prepared by David I. Shedroff, Enforcement Analyst, Office of Enforcement and General Counsel, Cincinnati Field Investigations Center, 5555 Ridge Avenue, Cincinnati, OH 45268.

Descriptors: Courtroom Procedure, Law Enforcement, Legal Aspects, Sampling, Water Analysis, Water Pollution Control, Water Quality Standards

Administrative & Court Proceedings, and Excerpts from Revised Draft of Proposed Rules of Evidence for the United States Courts can be found on the following pages.

ADMINISTRATIVE & COURT PROCEEDINGS

Court or Agency	Fact Finder	Burden of Proof	Comments
State Pollution Control Agency	Agency	As per statute - usually weight of evidence.	Hearing may be conducted by hearing examiner, agency member, or full agency. Appeal may be on facts and law or law alone, depending on statute.
Rule making-adjudication			
Federal Water Pollution Control Act			
Conference	Head of agency		Reports acceptable.
Hearing	Hearing Board		Specific testimony.
Court	Judge		Uses prior material, and may take additional testimony.
Court			
Civil Case -- - for money only	Judge or jury	Weight of evidence	
- injunction			
preliminary or temporary	Judge	Must show immediate harm or danger.	Must also show likelihood of success at final hearing - bond required for non-government plaintiff.
permanent	Judge	Usually clear and convincing.	"Balance Equities"
- administrative appeal	Judge - whether "arbitrary and capricious" or substantial evidence.		Sometimes have complete new trial.
Criminal case includes penalties	Jury unless waived.	Beyond reasonable doubt.	Proof of intent may be required.

Excerpts from Revised Draft of Proposed
RULES OF EVIDENCE FOR THE UNITED STATES COURTS

GENERAL PROCEDURES

Rule 102.
PURPOSE AND CONSTRUCTION

These rules shall be construed to secure fairness in administration, elimination of unjustifiable expense and delay, and promotion of growth and development of the law of evidence to the end that the truth may be ascertained and proceedings justly determined.

Rule 101.
PRELIMINARY QUESTIONS

(a) Questions of Admissibility Generally. Preliminary questions concerning the qualification of a person to be a witness, the existence of a privilege, or the admissibility of evidence shall be determined by the judge, subject to the provisions of subdivision (b). In making his determination he is not bound by the rules of evidence except those with respect to privileges.

(b) Relevancy Conditioned on Fact. When the relevancy of evidence depends upon the fulfillment of a condition of fact, the judge shall admit it upon, or subject to, the introduction of evidence sufficient to support a finding of the fulfillment of the condition.

Rule 615.
EXCLUSION OF WITNESSES

At the request of a party the judge shall order witnesses excluded so that they cannot hear the testimony of other witnesses, and he may make the order of his own motion. This rule does not authorize exclusion of (1) a party who is a natural person, or (2) an officer or employee of a party which is not a natural person designated as its representative by its attorney, or (3) a person whose presence is shown by a party to be essential to the presentation of his cause.

Rule 611.
MODE AND ORDER OF INTERROGATION AND PRESENTATION

(a) Control by Judge. The judge may exercise reasonable control over the mode and order of interrogating witnesses and presenting evidence so as to (1) make the interrogation and presentation effective for the ascertainment of the truth, (2) avoid needless consumption of time, and (3) protect witnesses from harassment or undue embarrassment.

(b) Scope of Cross-Examination. A witness may be cross-examined on any matter relevant to any issue in the case, including credibility. In the interests of justice, the judge may limit cross-examination with respect to matters not testified to on direct examination.

Rule 613.

PRIOR STATEMENTS OF WITNESSES

(a) Examining Witness Concerning Prior Statement. In examining a witness concerning a prior statement made by him, whether written or not, the statement need not be shown or its contents disclosed to him at that time, but on request the same shall be shown or disclosed to opposing counsel.

JUDICIAL NOTICE

Rule 201.

JUDICIAL NOTICE OF ADJUDICATIVE FACTS

(b) Kinds of Facts. A judicially noticed fact must be one not subject to reasonable dispute in that it is either (1) generally known within the territorial jurisdiction of the trial court or (2) capable of accurate and ready determination by resort to sources whose accuracy cannot reasonably be questioned.

(g) Instructing Jury. The judge shall instruct the jury to accept as established any facts judicially noticed.

RELEVANCE

Rule 401.

DEFINITION OF "RELEVANT EVIDENCE"

"Relevant evidence" means evidence having any tendency to make the existence of any fact that is of consequence to the determination of the action more probable or less probable than it would be without the evidence.

Rule 402.

RELEVANT EVIDENCE GENERALLY ADMISSIBLE; IRRELEVANT EVIDENCE INADMISSIBLE

All relevant evidence is admissible, except as otherwise provided by these rules, by other rules adopted by the Supreme Court, by Act of Congress, or by the Constitution of the United States. Evidence which is not relevant is not admissible.

COMPETENCY OF WITNESSES

Rule 601.

GENERAL RULE OF COMPETENCY

Every person is competent to be a witness except as otherwise provided in these rules.

Rule 602.

LACK OF PERSONAL KNOWLEDGE

A witness may not testify to a matter unless evidence is introduced sufficient to support a finding that he has personal knowledge of the matter. Evidence to prove personal knowledge may, but need not, consist of the testimony of the witness himself. This rule is subject to the provisions of Rule 703, relating to opinion testimony by expert witnesses.

EXPERT TESTIMONY

Rule 702.

TESTIMONY BY EXPERTS

If scientific, technical, or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training, or education, may testify thereto in the form of an opinion or otherwise.

Rule 703.

BASES OF OPINION TESTIMONY BY EXPERTS

The facts or data in the particular case upon which an expert bases an opinion or inference may be those perceived by or made known to him at or before the hearing. If of a type reasonably relied upon by experts in the particular field in forming opinions or inferences upon the subject, the facts or data need not be admissible in evidence.

Rule 705.

DISCLOSURE OF FACTS OR DATA UNDERLYING EXPERT OPINION

The expert may testify in terms of opinion or inference and give his reasons therefore without prior disclosure of the underlying facts or data, unless the judge requires otherwise. The expert may in any event be required to disclose the underlying facts or data on cross-examination.

Rule 706.

COURT APPOINTED EXPERTS

(a) Appointment. The judge may on his own motion or on the motion of any party enter an order to show cause why expert witnesses should not be appointed, and may request the parties to submit nominations. The judge may appoint any expert witnesses agreed upon by the parties, and may appoint witnesses of his own selection. An expert witness shall not be appointed by the judge unless he consents to act. A witness so appointed shall be informed of his duties by the judge in writing, a copy of which shall be filed with the clerk, or at a conference in which the parties shall have opportunity to participate. A witness so appointed shall advise the parties of his findings, if any; his deposition may be taken by any party; and he may be called to testify by the judge or any party. He shall be subject to cross-examination by each party, including a party calling him as a witness.

HEARSAY

Rule 801.

DEFINITIONS

The following definitions apply under this Article:

(a) Statement. A "statement" is (1) an oral or written assertion or (2) nonverbal conduct of a person, if it is intended by him as an assertion.

(b) Declarant. A "declarant" is a person who makes a statement.

(c) Hearsay. "Hearsay" is a statement, other than one made by the declarant while testifying at the trial or hearing, offered in evidence to prove the truth of the matter asserted.

Rule 802.

HEARSAY RULE

Hearsay is not admissible except as provided by these rules or by other rules adopted by the Supreme Court or by Act of Congress.

Rule 803.

HEARSAY EXCEPTIONS: AVAILABILITY OF DECLARANT IMMATERIAL

The following are not excluded by the hearsay rule, even though the declarant is available as a witness:

(5) Recorded Recollection. A memorandum or record concerning a matter about which a witness once had knowledge but now has insufficient recollection to enable him to testify fully and accurately, shown to have been made when the matter was fresh in his memory and to reflect that knowledge correctly. If admitted, the memorandum or record may be read into evidence but may not itself be received as an exhibit unless offered by an adverse party.

(6) Records of Regularly Conducted Activity. A memorandum, report, record, or data compilation, in any form, of acts, events, conditions, opinions, or diagnoses, made at or near the time by, or from information transmitted by, a person with knowledge, all in the course of a regularly conducted activity, as shown by the testimony of the custodian or other qualified witness, unless the sources of information or other circumstances indicate lack of trustworthiness.

(18) Learned Treatises. To the extent called to the attention of an expert witness upon cross-examination or relied upon by him in direct examination, statements contained in published treatises, periodicals, or pamphlets on a subject of history, medicine, or other science or art, established as a reliable authority by the testimony or admission of the witness or by other expert testimony or by judicial notice. If admitted, the statements may be read into evidence but may not be received as exhibits.

IDENTIFICATION OF PERSONS AND SAMPLES

Rule 901.

REQUIREMENT OF AUTHENTICATION OR IDENTIFICATION

(a) General Provision. The requirement of authentication or identification as a condition precedent to admissibility is satisfied by evidence sufficient to support a finding that the matter in question is what its proponent claims.

(b) Illustrations. By way of illustration only, and not by way of limitation, the following are examples of authentication or identification conforming with the requirements of this rule:

(1) Testimony of Witness with Knowledge. Testimony that a matter is what it is claimed to be.

(3) Comparison by Trier or Expert Witness. Comparison by the trier of fact or by expert witnesses with specimens which have been authenticated.

(9) Process or System. Evidence describing a process or system used to produce a result and showing that the process or system produces accurate result.

ADMISSIBILITY AND PROOF OF SPECIAL MATTERS

Rule 406.

HABIT; ROUTINE PRACTICE

(a) Admissibility. Evidence of the habit of a person or of the routine practice of an organization, whether corroborated or not and regardless of the presence of eye-witnesses, is relevant to prove that the conduct of the person or organization on a particular occasion was in conformity with the habit or routine practice.

(b) Method of Proof. Habit or routine practice may be proved by testimony in the form of an opinion or by specific instances of conduct sufficient in number to warrant a finding that the habit existed or that the practice was routine.

Rule 612

WRITING USED TO REFRESH MEMORY

If a witness uses a writing to refresh his memory, either before or while testifying, an adverse party is entitled to have it produced at the hearing, to inspect it, to cross-examine the witness thereon, and to introduce in evidence those portions which relate to the testimony of the witness.

Rule 1006.

SUMMARIES

The contents of voluminous writings, recordings, or photographs which cannot conveniently be examined in court may be presented in the form of a chart, summary, or calculation. The originals, or duplicates, shall be made available for examination or copying, or both, by other parties at a reasonable time and place. The judge may order that they be produced in court.

BIOASSAY AND BIOMONITORING

I INTRODUCTION

A An assay is an evaluation.

B A bioassay is an evaluation in which living organisms provide the scale.

1 The scale or degree of response may be the rate of growth or decrease of a population, colony, or individual; a behaviorial, physiological, or reproductive response, or simply a live or no-live response.

2 All types of bioassays may have a role to play in water quality evaluation at one time or another.

3 The particular group of bioassays discussed below are those which contribute to the evaluation of the effects of liquid wastes on aquatic environments in which experimental organisms such as fish are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions for a stipulated period of time.

C Historical Highlights

1 Prior to 1940, there was little or no uniformity in performing or reporting bioassays of water pollutants.

2 By mid-forties, the need for a standardized technique was becoming painfully obvious.

a The Atlantic Refining Company privately published the first statement of what is today, basically, our standard method (Hart, Doudoroff, and Greenbank, 1945).

b This was refined and accorded wide (but not universal) industrial, academic, and governmental acceptance in 1951 (Doudoroff, et al, 1951).

3 This method was first developed for the use of fishes, but has been found adaptable to a wide variety of organisms.

D Other Types or Plans of Bioassay

1 Many other designs for the expression of toxicity have been devised such as those based on time-concentration curves. Each has its advantages and proponents, but the basic Standard Method design remains the most widely used.

a In situ exposure of experimental organisms in cages or live cars, at selected sites above and below a suspected point or pollution is an obvious and time tested procedure, but lacks the precision of laboratory tests. It has the advantages of popular appeal, and of expressing actual environmental conditions.

b The familiar BOD test is a bioassay of the organic content of water subject to biodegradation.

E Biomonitoring

Water quality surveillance or monitoring by means of observing biota can be considered from two aspects: field and laboratory. It differs from bioassay primarily in the objective: a bioassay is an attempt to determine a specific defined value or threshold, whereas a biomonitoring operation is an attempt to use living organisms to ascertain whether or not aquatic life is endangered.

1 Periodic biological field surveys, samples, or other observation may demonstrate recent excessive pollution for example.

2 Organisms in a series of flow-through tanks in a laboratory may demonstrate the occurrence of an unacceptable

increase in the toxicity of an effluent, without measuring "how much" or "what."

II THE STANDARD METHOD BIOASSAY

A Introduction

1 This procedure is intended for use by industrial and other laboratories.

2 Its objective is to evaluate the toxicity of wastes and other water pollutants to fish or other aquatic organisms.

3 Potential applications are numerous.

a Dilution and/or treatment necessary to avoid acute toxic effects can be estimated.

b The efficacy of an existing treatment can be tested.

c The potential usefulness of a proposed treatment can be estimated.

4 The design of the test need not involve a chemical knowledge of the toxicant.

a Synergism, antagonism, and other interactions of chemical components cannot always be anticipated, but are automatically included in the result.

b All chemical and physical information available is, however, essential to the adequate interpretation and application of test results.

5 The test is best used for local application. Generalizations should be made with great caution.

6 Field observations should be made of results of application over a significant period of time.

7 Careful distinction should be made between fish mortality due to a physiological toxicant, and that due to lack of DO.

8 A uniform testing procedure is essential to effective action in water pollution control.

B Routine Procedure for Static Tests

1 Test organisms should be fish or other organisms of local significance.

a The most sensitive species available should be selected, but:

b They should be species which are amenable to captivity.

c They should be accurately identified.

d They should be relatively uniform in size. Individuals less than 3 inches in length are usually most convenient.

e They should be healthy and thoroughly acclimated to the laboratory.

f A careful record should be kept of their origin, handling, and condition.

2 Test water should preferably be taken from the receiving stream just above the discharge being evaluated or in a lake or estuary, beyond the influence of the discharge.

a If this is unsuitable, cleaner but similar waters from a more remote station may be substituted.

b Artificial "standard" waters are not recommended for general use, although many formulae have been proposed.

c In estuarine situations, a series of tests (marine grid) should be run, using waters of high and low salinities as characteristic of the region.

3 Other experimental conditions

a **Temperature.** The tests should be performed at a uniform temperature in the upper part of the expected summer range, e.g., 25 ± 2°C for warm water fish, and 15 ± 2°C for cold water species.

b Test containers should be of glass, widemouthed "pickle jugs" or battery jars are satisfactory. Five and one gallon sizes are both useful, but the larger size is required for conclusive results.

c Artificial aeration should not be used to maintain the dissolved oxygen concentration. If this falls below approximately 4 or 5 ppm at any time during the test, fewer fish should be used per container or an auxiliary oxygenation procedure invoked that is designed to avoid undue loss of volatile toxicants.

d The number of test animals should not be less than 10 per concentration for reliable conclusions; these may be distributed between two or more containers.

e Ratio of fish to solution. There should be not more than one gram of fish per liter of test solution.

4 Experimental procedure

a All dilutions for a given run should be prepared from the same sample.

b Duration. Tests should be run for at least 48 hours, preferable 96.

c Dead fish should be removed as soon as observed. Survivors should be counted and recorded each 24 hours.

d Feeding during the test should be avoided.

e Experimental concentrations. Any appropriate series of concentrations may be used. A logarithmic series such as is suggested in Table I is very convenient.

TABLE I

A Guide to the Selection of Experimental Concentrations, Based on Progressive Bisection of Intervals on a Logarithmic Scale.

Col. 1	Col. 2	Col. 3	Col. 4	Col. 5
10.0	----	----	----	----
----	----	----	----	8.7
----	----	----	7.5	----
----	----	----	----	6.5
----	----	5.6	----	----
----	----	----	----	4.9
----	----	----	4.2	----
----	----	----	----	3.7
----	3.2	----	----	----
----	----	----	----	2.8
----	----	----	2.4	----
----	----	----	----	2.1
----	----	1.8	----	----
----	----	----	----	1.55
----	----	----	1.35	----
----	----	----	----	1.15
1.0	----	----	----	----

Effluents of unknown, mixed, or variable composition are usually best expressed as percent by volume; while pure substances, or specific analyzable components are usually expressed as milligrams per liter (ppm). A control or reference tank containing dilution water only (with no toxicant) is essential, to demonstrate that all experimental organisms would have survived had it not been for the toxicant being tested.

f Expression of results. The measure of relative toxicity is the tolerance limit (symbol: TL). The time of exposure "t" must be shown along with the percentage of fish surviving (written as a postscript). For example, a 96 hour TL50 (optional: TL50$^{96\ hr}$) of a toxic substance is that concentration in which 50% of the experimental organisms survive for 96 hours. (Figure 1)

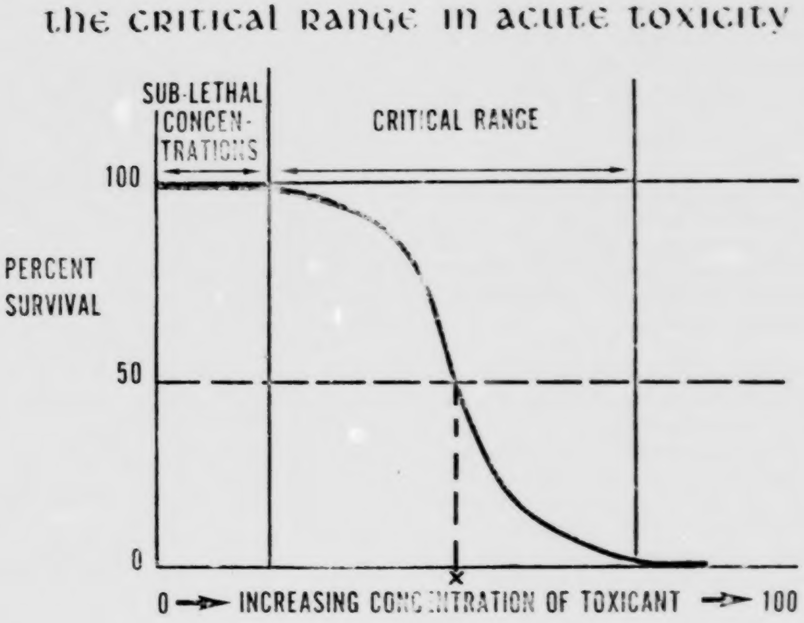

Figure 1
THE CRITICAL RANGE
X = $TL50^t$ Concentration

1) A $TL50^t$ is the equivalent of a median tolerance limit (TL_m^t).

2) This is analogous to the LC_{50} (concentration survived by 50% of the population) of the toxicologist, but is more universally usable with the parameters encountered in the water environment, some of which (such as temperature) cannot be expressed as "concentrations."

3) TL50's for 96 hours or less are arbitrarily referred to as measures of "acute" toxicity, while TL50's for longer periods of time are variously referred to as sub-acute, chronic, etc.

C Special Problems of Static Tests

1 Unaerated aquaria with finite quantities of toxicant are not always satisfactory (static tests).

a The toxicant may be volatile.

b Toxic materials may be masked by a high BOD.

c The toxicant may be progressively adsorbed on container walk, fish slime, metabolized or otherwise changed so that actual concentrations in tanks change with time.

2 Standards or requirements other than those involving toxicity per se may be involved.

3 Preliminary and concurrent investigations

a Obtain all available information about unknown to be tested.

b Does the material lend itself to this type of test?

c Run feasible on the spot analyses including DO.

d Significant quantities of solutions removed from test containers for analysis should be replaced with similar volume of same dilution.

4 Wastes with a high BOD or COD

a Suggested preliminary tests:

1) Set up two identical exploratory tests.

2) Aerate one but not the other.

3) If great difference develops between them, special procedures are indicated.

b Oxygenation or aeration of dilution water before making dilutions may help.

c Renewal of solutions at stated intervals (12, 24, or 48 hours) is approved. Fish are not harmed by being carefully transferred from one container to another. It is useful where:

1) Initial DO is adequate but slowly exhausted.

2) Toxicant is volatile, progressively adsorbed, precipitated, or otherwise changed.

D Continuous Flow Procedures

1 Continuous flow procedures imply the continuous or periodic renewal of the solutions in the experimental containers, at the same time maintaining the stated concentrations (including control). The variety of devices and flow plans to accomplish this are almost infinite, two general principals will be outlined below: assaying and monitoring.

2 Continuous flow bioassay, general advantages (Figure 2)

a Materials with moderate oxygen demands may be tested.

b Materials which degrade or are volatile may be tested.

c Due to the constant removal of metabolic and other wastes, and the constant supply of fresh oxygenated water, fish may be fed and so maintained over a longer period of time. Containers must of course be maintained in reasonably clean condition.

E Test Concentrations and End Points for Continuous Flow Assays

1 Test concentrations are in general less restricted than for static tests. They need not be so high as to insure achieving the desired end point in 48 or 96 hours, although they may be so set if desired.

2 Geometric type series of concentrations are still desirable (See Table 1).

3 Sub-lethal levels may be tested over entire life histories of organisms to determine long range effects.

4 In general, the setup should be prepared, calibrated and operated for several days, or until the concentrations have become chemically and physically stabilized before introducing the fish or other experimental organisms.

F Total Fish Weight and Liquid Volume in Continuous Flow Assays

In general, the constant renewal of test solution might appear to make possible testing more or larger fish in less water. Actually, flow-through volume and total weight of fish must be so related that adequate oxygen is maintained. Furthermore, over the longer periods of time involved, "lebensraum" (or territory) must be taken into account. Organisms must not be crowded to the extent that aggressive behavior and other ecological competitive factors are introduced.

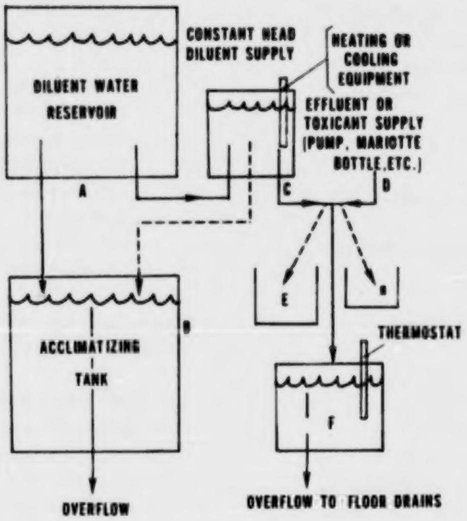
CONSTANT HEAD DILUENT SUPPLY
HEATING OR COOLING EQUIPMENT
DILUENT WATER RESERVOIR
EFFLUENT OR TOXICANT SUPPLY (PUMP, MARIOTTE BOTTLE, ETC.)
A
C
D
E
n
B
ACCLIMATIZING TANK
THERMOSTAT
F
OVERFLOW
OVERFLOW TO FLOOR DRAINS

Figure 2

BASIC SETUP FOR CONTINUOUS FLOW BIOASSAY

E to n represents one of several exposure tanks containing a graded series of dilutions of the toxicant, including one control with none.

G End Points or Reactions to be Evaluated by Continuous Flow Assays

1 The original and traditional end point of biological evaluations such as those discussed here was the death of the organism. This was simple, direct, and unequivocal. Current practice, however, often involves much more sophisticated reactions such as reduction in the reproductive capacity, or a change in the breathing rate (movement of gills).

H Special Problems of Flow Through Bioassays

1 Due to the physical requirements of maintaining stated concentrations of chemicals over long periods of time, laboratory setups are usually complicated and always require attention and maintenance.

2 The problem of disease control frequently develops in populations held over a long period of time.

3 Water and/or power failure may jeopardize an assay experiment after months of time have been expended.

4 The expense of a long continued test may not be justified by the result.

5 The above points demonstrate that in general, flow through bioassays are not adapted to day-to-day routine toxicity determinations.

III REPORTING INTERPRETATION AND APPLICATION OF BIOASSAY RESULTS

A Reporting

1 Reports should include an orderly tabulation of all pertinent data such as:

a The type of setup used and duration of test

b Identity of experimental animals

c Their source, history, average size and condition, and number used per concentration

d Source of, and chemical and physical analysis of experimental dilution water

e Experimental temperature

f Volumes of experimental liquid in each container

g Records of routine analyses such as DO and pH

h Records of chemical analyses of toxicants in experimental tanks

i TL50^t or other end point, and data from which it was determined.

B Interpretation and Application

1 The TL50^t is an estimate of the mid-point of the critical concentration range the interval between the highest concentration at which all test animals survive, and the lowest at which they all die (Figure 1).

2 The final step is to extrapolate from this well established mid concentration to a safe concentration well below the "critical concentration range." Extrapolating or rather: "application factors" to accomplish this are still under development and will probably not be fully developed for many years. Available data indicate that these factors must be variable according to the toxicant in question acting in combination with the receiving water in question, and considering the entire aquatic community.

3 For a more complete discussion, see FWPCA Water Quality Criteria (Reference No. 9) pp. 55-72.

4 Other considerations

a Radioactive wastes must be evaluated with regard to their chemical toxicity as well as their radioactivity.

b Sub-acute levels of many toxicants such as lead, arsenic, cadmium, etc. may exert a low level chronic toxicity over a long period of time.

c "Safe levels" of a waste in regard to toxicity to aquatic life may still exceed standards of other types such as color, organic content, suspended solids, etc.

IV BIOMONITORING AS COMPARED TO BIOASSAY (Figure 3)

A Bioassay is (as stated above) the evaluation of the effects of stated concentrations of the test material for given periods of time.

B Biomonitoring is the use of organisms to detect change in an effluent (surveillance). It operates continuously and indefinitely.

C Bioassays typically involve relatively small flows and employ often especially prepared (perhaps repeatedly prepared) batches of experimental material, while biomonitoring typically involves larger flows, from operating industries or other installations.

D Bioassays basically determine:

1 Is the substance deleterious, and if so:

2 How deleterious is it?

E Biomonitoring is useful to

1 Demonstrate the continuous suitability of an effluent (or a predetermined dilution thereof) for the survival of the test organism.

2 Detect a change (usually deleterious) in the biological acceptability of the effluent.

3 To detect a change in the effect of a mixture of the effluent and the receiving water on the test organism (i.e. to detect a change in the receiving water).

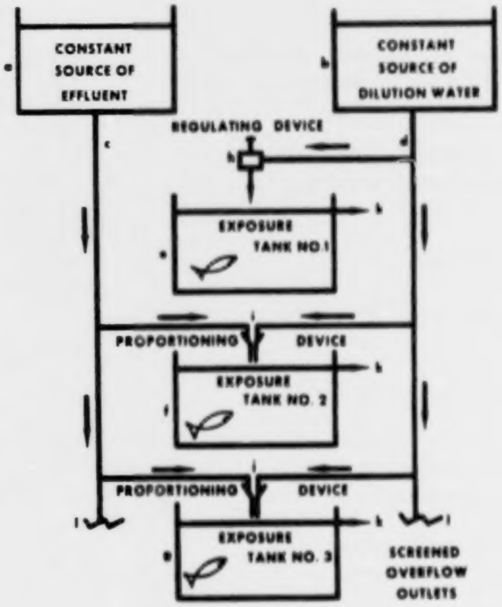
a
CONSTANT SOURCE OF EFFLUENT
b
CONSTANT SOURCE OF DILUTION WATER
REGULATING DEVICE
c
d
h
EXPOSURE TANK NO. 1
e
PROPORTIONING DEVICE
EXPOSURE TANK NO. 2
f
PROPORTIONING DEVICE
EXPOSURE TANK NO. 3
g
SCREENED OVERFLOW OUTLETS

Figure 3
BASIC SETUP FOR BIOMONITORING

F Biomonitoring was originally effective only with relatively fast acting materials, or in situations where large changes might occur rather quickly (as for example, the accidental (?) dumping of a vat of waste pickle liquor). Recent developments in the field of biotelemetry now make it feasible to "wire" a fish with electrodes (like the astronauts) and so to immediately record electronically any sudden or subtle change in the effluent which affects the physiological parameters being monitored on the live fish.

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This outline was prepared by H. W. Jackson, formerly Chief Biologist with the National Training Center, Water Program Operations, EPA, Cincinnati, OH 45268.

BIOASSAY FACILITIES AND EQUIPMENT

I INTRODUCTION

A Types of organisms and where they can be obtained are discussed elsewhere.

B Here we are concerned with facilities and equipment for working with the test animals.

II EXTENT OF FACILITIES AND EQUIPMENT NEEDS

A Depend on Several Considerations

- 1 Number and size of test animals
- 2 Type of study
    - a Static vs continuous-flow-through
    - b Death-survival vs autopsy-sublethal effects
    - c Laboratory setup vs outdoor setup
- 3 Space limitations
- 4 Budget and staff available or planned
- 5 Extent of bioassay program

III STATIC OR CONTINUOUS FLOW

A Static Studies Suitable for:

- 1 Screening tests for "ball park" toxicity values to be used in long-term testing.
- 2 Comparative toxicity of compounds having similar metabolizing qualities.
- 3 Comparative toxicity of various process effluents in an industrial operation.
- 4 Screening organisms for relative sensitivity to a given toxicant.
- 5 When the available toxicant is in limited quantity it is sometimes necessary to use a static bioassay.

B Advantage of Static Test

- 1 Simplest to set up

C Shortcomings of Static Test

- 1 Animals are bathed in their own waste materials, some of which are toxic.
- 2 Many toxicants decay with time, floc, or precipitate, resulting in lower than desired concentrations.
- 3 Some test organisms can absorb much of the toxicant into their tissues and reduce concentrations in water.

D Situations Which Should be Analyzed by Using a Continuous-Flow Setup

- 1 TL_m determinations in general, exceptions only for extremely short-term tests.
- 2 Any test in which the size or hardiness of the test organisms compared to the volume of the test chamber suggests problems of waste products buildup or potential diminishing concentrations of toxicant.
- 3 Long-term tests studying effects of continuous or periodic exposures.

E Shortcomings of Continuous Flow

- 1 Requires more space, time, and equipment.

F Advantage of Continuous-Flow-Through

- 1 More accurate results.

IV NECESSARY FACILITIES AND EQUIPMENT

A For Static Bioassay

- 1 Discussed elsewhere

B For Continuous-Flow Bioassay

1 Test chambers - glass, fiberglass, plastic, and stainless steel.

2 Dosage apparatus for adding toxicant, including gear pumps, constant-level-float siphons, Mariotte bottles, dipping bird gadgets, syringe devices, and various combinations of adjustable-volume venturi-siphon units.

3 Water flow control devices, including adjustable headboxes, constant level float valves, simple shut-off valves, capillary or tapered glass tubing, and adjustable-volume venturi-siphon units.

4 Combination units handling both toxicant and water.

a "Slurp-chamber" apparatus

b Serial diluters

c Simplified automatic dosage apparatus.

C For Special Application

1 Mount degasser

2 Temperature control devices and recorders

3 pH controlling unit and recorders

4 Demineralizers and carbon filters

5 Paddle wheel setup

6 Variety of test chambers

7 Swimming ability apparatus

8 Movement detectors and recorders

9 Egg collecting and hatching apparatus.

D Additional Items Needed or Useful

1 Necessities include: air pumps or compressed air system, plastic air tubing, glass and brass fittings for air tubing, air stones, small clamps, variety of plastic and rubber tubing, variety of regular and capillary glass tubing, rubber stoppers, formalin or alcohol preservative, chemical laboratory glassware (including pipets, graduate cylinders, etc.), fish-holding tanks for reserve specimens, small dip nets, food, data recording form stamps, pipet bulbs, water quality analyzing equipment (for DO, pH hardness, alkalinity, etc.), Toxicant analysis equipment (colorimeter, polarograph, chromatography setup), portable aerating equipment, fish and water transporting containers, fish treating compounds (antibiotics, parasite control chemicals), refrigeration facilities.

2 Items with special application: Dissecting equipment, tissue processing material (for fixing, embedding, and staining), microtome, microscopes, drying and ashing ovens, vacuum pump, scales and balance, activated carbon column, plankton counting equipment, appropriate text books and manuals.

E Space Requirements in General

1 Area for holding fish for future tests

2 Area for test chambers

3 Area for diluent water storage

4 Area for dosage apparatus

5 Area for water quality and toxicant analysis equipment

6 Sink and drainboard space

7 General storage area

8 Additional space for special equipment.

F Example of a Static Bioassay Laboratory (see Figure 1)

1 Area Number 1, provides space for holding and acclimatizing fish. Each

large aquarium for holding fish (A) is adequate for accommodating about 200-300 average size test speciments. An adequate air supply must be available at all times to provide for continuous aeration. Aquarium filters (C) of the inside type help in keeping the aquarium clean but best results come from continually trickling fresh water into the holding tank. Ordinary tap water can often be used for holding fish if chlorine has been removed, for example, by passing the water through an activated carbon column (E) and temperature changes are not too abrupt. The smaller aquarium (B) may be used for acclimating the test fish to the experimental water or holding them without food for period immediately preceding the tests.

2 Area Number 2 provides for storage and preparation of dilution water. While containers for hauling and storage may be of other inert materials, size, and shape, the polyethylene items illustrated (L, M) have been very satisfactory for this use. In addition

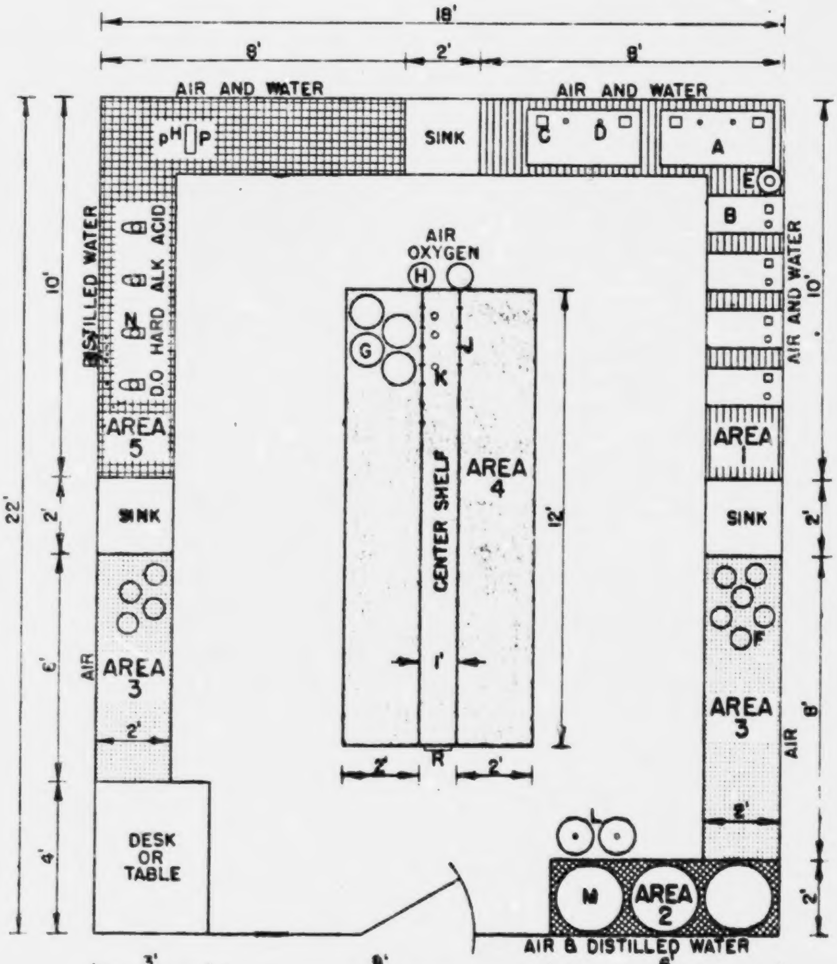
18'
8'
2'
8'
AIR AND WATER
AIR AND WATER
pH P
SINK
C
D
A
E
B
AIR
OXYGEN
H
G
K
J
CENTER SHELF
AREA 4
12'
DISTILLED WATER
N
D.O.
HARD
ALK
ACID
AREA 5
10'
22'
SINK
2'
AIR
AREA 3
6'
2'
DESK OR TABLE
4'
1'
R
2'
2'
AIR AND WATER
10'
AREA 1
SINK
2'
F
AREA 3
8'
AIR
2'
L
M
AREA 2
2'
AIR & DISTILLED WATER
3'
9'
6'

FIGURE 1

to the regular supply it may be desirable to have a supply of distilled or demineralized water.

3 Area Number 3 can be used for preparing experimental test concentrations of the effluent in the dilution water and for exploratory tests to determine the approximate toxic range. In these tests air may be needed, depending on the nature of the effluents.

4 Area Number 4 supplies bench space for holding 20 full-scale test chambers (G) on each side which permits the carrying on of at least 4 full-scale bioassays simultaneously. Air must be provided. A convenient arrangement for supplying air or oxygen is through a system of small tubing and 3-way air valves so that the supply to each test aquarium can be regulated independently. This system may be attached either to the air supply or to an oxygen cylinder (H) equipped with a pressure reduction valve and regulator.

5 Area Number 5 is for conducting the necessary chemical tests for oxygen control and to provide information necessary for interpreting bioassay data. Squeeze-o-matic burettes (N) have been found quite useful for rapid performance of certain chemical tests.

V DILUENT WATER

A Type of diluent water depends on the type of data sought.

1 Fresh, brackish, or salt water - depends on organisms.

2 If interested in the effect of a particular compound in a particular water body (stream, lake, estuary, etc.) then use water from the location where this compound would enter the waterway.

3 Generally for other purposes, the best water is the one you have most readily available. Tap water can be dechlorinated; spring, stream, or pond waters are usually suitable. Well water is not generally recommended, however.

4 By mixing water from two sources in large aerated storage facilities, close water quality can be maintained.

This outline was prepared by T.O. Thatcher, Former Aquatic Biologist, Research and Development, Cincinnati Water Research Laboratory, FWPCA.

IMPORTANT DATA FROM ACUTE MORTALITY TESTS

I MEDIAN LETHAL CONCENTRATION (LC50)

A Mortality

The major result of an acute mortality test with a particular toxicant and a certain species of fish is the LC50, ie, the concentration that kills half of the fish. In order to calculate the LC50, one must know the percent mortality for a series of concentrations of the toxicant. Determining the percent mortality merely involves counting the live and dead fish, but one should report the criteria for determining whether a fish is live or dead.

B Concentration of Toxicant

Measuring the concentration of the toxicant is a more difficult problem. In some cases researchers do not measure the concentration of the toxicant. This is especially true of toxicity tests conducted with complex mixtures. In these cases, the results are calculated based on the amount of toxicant that was supposed to have been used. (The calculation of an LC50 from the mortality vs. concentration data will be covered in a later lecture).

In most cases the concentration of toxicant in the water is measured. However, it is not always easy to decide what measurement to make. Consider the following cases:

1 If a small amount of DDT is put into a jar of water about 70 percent will absorb to the glass walls of the jar, about 30 percent will accumulate at the air-water interface, and about 10 percent will dissolve in the water. Should one base an LC50 calculation on the total amount of DDT in the jar or on the concentration of DDT dissolved in the water?

2 If a moderate amount of copper sulfate is put into a jar of water, about 20 percent will dissolve and about 80 percent will become a basic copper precipitate. Some of the precipitate will form a scum on the water and the rest of it will be distributed throughout the water, probably with most of it on the bottom of the jar, depending on how well the fish stir up the water. Should one calculate the LC50 based on the total amount of copper in the jar, the dissolved copper, or the amount of copper dissolved and suspended in the water? The answer to this question must take into account the fact that some species of fish spend most of their time near the surface and some spend most of their time sitting on the bottom.

3 If phenol is placed in the water, it will exist both as unionized phenol and as ionized phenate ion. It is possible that one form is much more toxic than the other. However, practically all methods for measuring phenol in water will measure the total amount of phenol in the water.

These examples indicate that one must decide exactly how the sample must be taken and what analytical methods can be used. Very often the use of the results of the toxicity test will determine the answers to these questions. Usually for static bioassays the LC50 calculation is based on the total amount of toxicant put in the test container at the beginning of the test. This procedure obviously has its drawbacks. For continuous-flow tests, generally the best approach is to take a sample under the surface of the water. Because of the constant mixing in the test chamber, this usually, but not always, represents the total concentration of toxicant to which the fish are exposed.

The decision as to how the sample should be collected and/or what measurement should be made must depend on what question the test is supposed to answer. The two common questions are:

1. How much toxicant must be added to the water to affect the fish in a certain way?

2. How much toxicant must the fish be exposed to in order to affect them in a certain way?

C Calculation of the LC50

In reporting the results of a toxicity test one should report the LC50 for a given length of exposure with its confidence limits, the way the concentration of the toxicant was determined, and the method used to calculate the LC50 from the concentration-mortality data. Some people report the concentration-mortality data itself.

II OTHER INFORMATION

A About the Fish

There is much other information about an acute mortality test that should be reported along with the LC50 value. This can be broken down into four categories. One should report information about the fish, the test conditions, the toxicant, and the water used in the test. This information is important because there are many things that can affect the LC50, and unless this information is reported, no one else can use the data. Under information about the fish, one should report both the scientific and common names, the age, life stage, sex of the fish, and the range of the lengths or weights or both. Very often people report where the fish were obtained and their condition, any treatments used on them, and the holding and acclimatization procedures used.

B About the Physical Setup

Information on the physical setup should include the type of test chambers used, the volume of water used, the number of fish per test chamber, and the average grams of fish per liter of water and experimental design. For continuous-flow tests one should report the flow rate.

C About the Toxicant

Information about the toxicant should identify the source of the toxicant and its composition. One should also describe the formulation of stock solutions used to introduce the toxicant into the test chambers.

D About the Water

Information about the water should include the pH, alkalinity, dissolved oxygen, hardness, total dissolved solids, and temperature. Conductance and acidity may be useful. Calcium, magnesium, sodium, potassium, chloride, and sulfate measurements can help characterize the water. Many people also report the source of the water and any pretreatment, such as aeration, activated charcoal, or softening. One should also measure and report any unusual constituents in the water, constituents present in unusual amounts, or constituents which are known to affect markedly the toxicity of the material under test.

Once you have decided what measurements to make, it is important to use a good method for the determination. One is actually better off having no information rather than having wrong information. I would recommend use of methods from the EPA manual titled "Methods for Chemical Analysis of Water and Wastes" whenever possible. The manual has two basic purposes:

1. To identify the simplest possible legally defensible methods;

2. To promote standardization so that results will be comparable from one laboratory to another.

VII OTHER TOXICITY TESTS

The same kinds of information should be reported for any other kind of toxicity test with any other test organism.

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This outline was prepared by C. E. Stephan, Supervisory Research Chemist, Newtown Fish Toxicology Laboratory, Newtown, OH 45244.

BIOMONITORING OF INDUSTRIAL EFFLUENTS

I INTRODUCTION

A Plant operating personnel need to know the general quality of an effluent which is being discharged at a fairly constant rate and also must be warned if a slug of toxic material is released to the receiving water.

B Conventional bioassay procedures can evaluate only single samples taken at particular times. Continuous-flow bioassays of single grab samples over a long period of time can be very useful, but do not solve the problem of transient variations.

C A technique that does permit exercising continuous surveillance over the toxicity of an effluent is biomonitoring.

D Conventional bioassays (2, 3, 4) can provide important information about the actual toxicity of batches of the effluent in terms of TL_m's, and, if sufficient samples are tested, about the range of variation. Bioassays should be run from time to time to ascertain the exact toxicity of a waste even though it is being monitored as outlined below.

E The following procedures refer only to toxic wastes having a relatively rapid action. Wastes such as cadmium which have long delayed cumulative effects at low concentrations, (5) oxygen-demanding wastes, radioactive wastes, and others, would either be inappropriate or would not elicit a recognizable reaction soon enough to be of use.

II OBJECTIVES OF BIOMONITORING

Three basic objectives of biomonitoring are:

A To demonstrate the continuous suitability of an effluent for aquatic life provided slow-acting or cumulative toxins are not involved. The continuous testing of an undiluted effluent, (Objective A) is usually accomplished by leading a small stream of the effluent through an aquarium. This aquarium may be located in a public lobby to enhance public relations, or it may be in the plant for operational use only. This is a relatively simple and direct approach and needs no further elaboration.

B To detect change (usually deleterious) in the biological acceptability of the effluent itself.

C To detect change in the effect of the effluent on the biota of the receiving water.

III EQUIPMENT

A single basic design of exposure tanks and flow plan can be used to accomplish Objective B or C. (Figure 1) With the exception of a simple suggestion for proportioning flow of effluent to dilution water (Figure 2), engineering devices for accomplishing the various needs outlined below are not discussed. (6, 7, 8) Special care should be used to ensure that all surfaces that come in contact with the waste or the dilution water are constructed of nontoxic and noncorrosive materials. This precaution is particularly necessary for marine waters, where bimetallic contacts are very dangerous. An experienced aquatic biologist should be consulted in the preparation of plans.

A Exposure Tanks

1 Exposure tanks (Figure 1) should be large enough (10 to 20 gallons) that the test organisms can live normally under plant conditions. (3) Simple construction

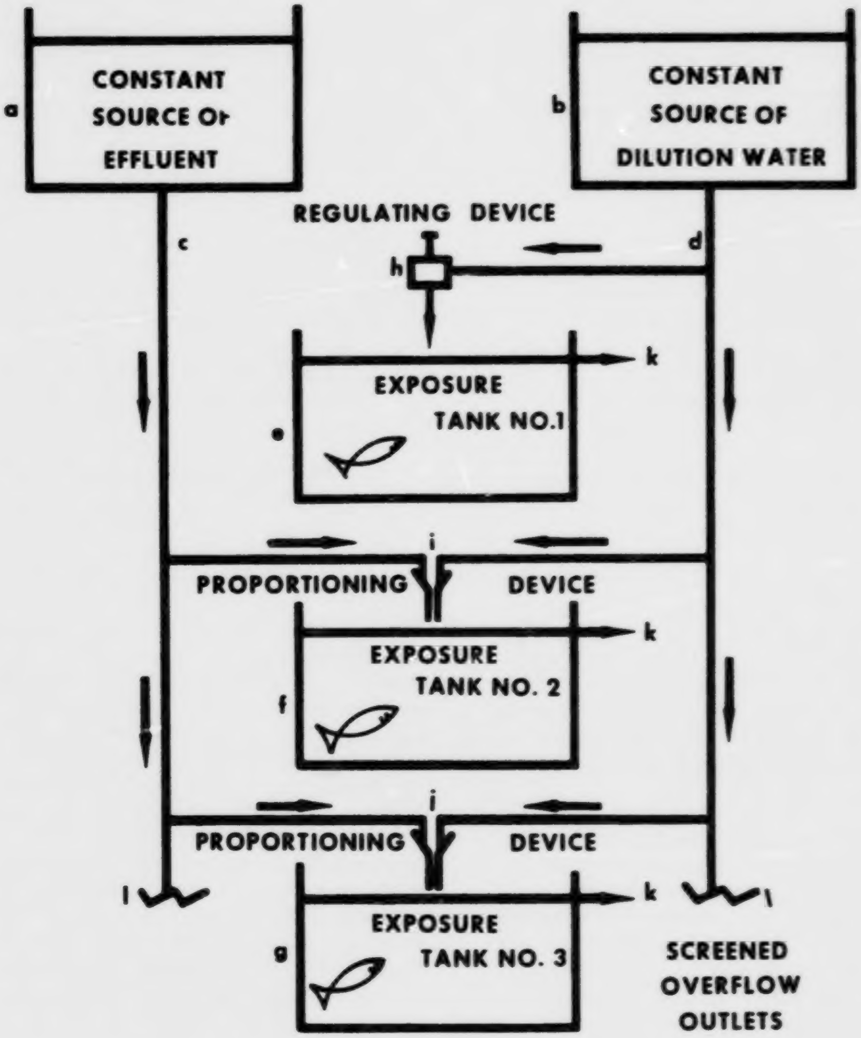
a
CONSTANT SOURCE OF EFFLUENT
b
CONSTANT SOURCE OF DILUTION WATER
REGULATING DEVICE
c
d
h
k
EXPOSURE TANK NO.1
e
i
PROPORTIONING DEVICE
k
EXPOSURE TANK NO. 2
f
i
PROPORTIONING DEVICE
l
k
l
EXPOSURE TANK NO. 3
g
SCREENED OVERFLOW OUTLETS

Figure 1. SCHEMATIC FLOW PLAN FOR OBJECTIVES B OR C

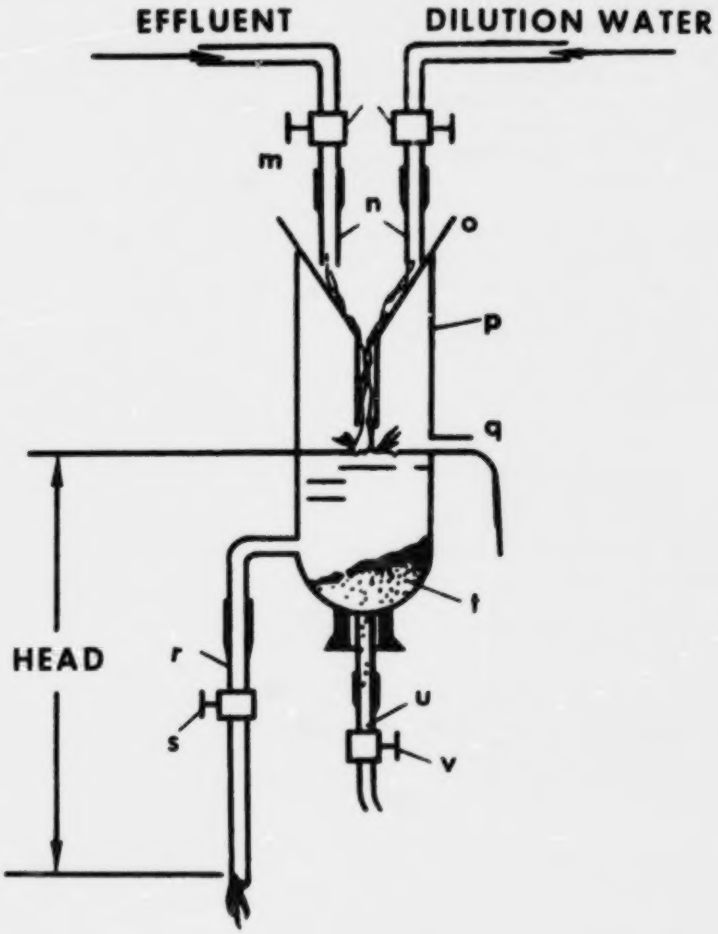
EFFLUENT
DILUTION WATER
m
n
o
p
q
t
HEAD
r
s
u
v

Figure 2

SUGGESTED PLAN FOR PROPORTIONING FLOW BETWEEN EFFLUENT AND DILUTION WATER. (See i and j, Figure 1) will facilitate feeding, cleaning, and disease control. "Eye-appeal" is not necessary unless public relation is involved, but scrupulous sanitation is essential as in all long-term animal culture.(9, 10) Tanks should be situated in a lighted and well-ventilated room, but not exposed to direct sunlight. Ambient room temperatures are generally satisfactory.

2 Inlet and overflow should be similar in all tanks so that conditions will be the same except for the quality of the water. The total flow of water through the tank should be adjusted so that the hydraulic retention times are equal, whether the total flow is coming from one source or two.

3 Tank No. 1 contains unadulterated dilution water to establish that the test animals will live in it. This tank is the control or "reference" to which the other tanks are compared.

4 Tanks 2 and 3 (more may be added at points 1) contain mixtures of effluent and dilution water. If the experimental animals die or show distress in these tanks, a change for the worse in the characteristics of the effluent being monitored is probably indicated.

5 i and j are mixing or proportioning devices set to predetermined amounts.

6 All tanks overflow to the sewer through opening k, which should be screened to prevent escape of test animals and clogging in outlet pipe.

B Effluent Supply

The supply of effluent should be constant and controllable. The prime requisite is that it be fresh so that changes may be detected at the earliest possible moment.

C Dilution Water

1 The source and quality of the dilution water is the most important single factor in the system because this is the scale or standard by which the toxicity of the effluent is assayed.

2 To test the effect of the effluent on the receiving water (Objective C), the dilution water obviously must be the receiving water.

a In most cases the receiving water varies from time to time as runoff water washes in different materials from the surface of the land the tide turns, or other plants release various wastes.

b Plan C (Objective C) automatically evaluates the toxicity of the effluent when discharged into this changing situation. This water should contain all of the components present down to the outfall being monitored, but none of the effluent itself.

3 In a stream situation, dilution water can be taken well upstream from the waste discharge. A pipeline with a continuous flow is ideal, since slugs of material from upstream sources that might modify the toxicity of the effluent being monitored would be taken into the monitoring system and quickly distributed to the exposure tanks. Because such systems are notoriously subject to "problems," batch transportation of control water may have to be employed. In lakes, estuarine, or coastal situations, batch supply may be the only practical solution.

4 If the receiving water is already continuously toxic to aquatic life, it is unsuitable for use in the system.

a Under such circumstances Plan C would be unworkable and the only recourse would be Plan A or B.

b Treated water such as drinking water from a city or industrial plant should never be used for any of these plans, even if dechlorinated, because the chemicals used in treatment may react with the waste being monitored.

D Proportioning Flow

The plan (Figure 2) by which effluent and dilution water are proportioned to the test tanks (Tank 2 and 3) determines what the system will accomplish.

1 A suggested plan for proportioning flow between effluent and dilution water (i and j) in Figure 1 is given in Figure 2.

2 Rubber tubes (n) can be momentarily diverted to catch flow in an appropriate sampling device such as a graduated cylinder. Based on the time required to discharge some standard volume, flow could be proportioned to any desired ratio (for example, one part effluent to two parts dilution water).

3 The removal of sediment by some sort of trap as shown is a policy matter that should be resolved in the project-design statement.

IV FLOW PLANS

A Flow Plan for Objective B

If the system is to be operated to detect only serious detrimental change in the effluent itself, Tanks 2 and 3 should contain mixtures in such proportions of effluent and dilution water as to permit the test animals to live as long as the effluent is normal.

1 One possible combination would be to adjust the mixing mechanism at i (Figure 1) to admit such a proportion of effluent that the test animals in Tank No. 1 could barely survive. The slightest increase in toxicity of the effluent would then immediately be made evident by the death of the test animals and appropriate remedial action could be taken.

2 The mixing mechanism j in Tank No. 3 might be adjusted to provide a greater margin of safety, for example 1/2 or 1/10th the toxicity of Tank 2. If Tank 2 animals then died, but Tank 3 animals survived, it would presumably indicate only a moderate increase in toxicity.

B Flow Plan for Objective C

Monitoring the effect of the effluent on the receiving water, from the point of view of protecting aquatic life, is ideal, but may also be very difficult.

1 The reference tank (e, Figure 1) receives water fresh from the receiving body. This water is free of any trace of the effluent being monitored, but contains all substances, natural and artificial, presently in the receiving water. These components may change from time to time, and one of these changes may increase the toxic effect of the effluent (synergism).

Thus the death of animals in the strongest test tank, but not in the reference tank, may be the result of an increase in the toxicity of the plant waste, or of a synergistic reaction of the effluent with a material in the receiving water.

2 No matter the cause, the death itself serves as a warning and immediate action can be taken on the discharge to protect aquatic life.

3 On the other hand, if a slug of strongly toxic matter enters the receiving water from some outside source, the deaths of the animals in the reference Tank 1 (and probably also those in exposure Tanks 2 and 3) would show that the "fish kill" presumably in progress in nature was not the result of the effluent.

4 A parallel installation under Plan B might also demonstrate no change in the waste being monitored.

5 Numerous other dilution systems might also be employed under Plan C.

C A different approach is to proportion the mixture to simulate the actual mixture taking place in the receiving water. For this purpose if the location were on a flowing stream, the flow of both the stream and final effluent must be known. Periodic adjustments might be made by hand, or by automatic equipment involving telemetry of both effluent and stream flow.

V TEST ANIMALS AND OTHER CONSIDERATIONS

No universal recommendations can be made about test animals to be used although many suggestions are available.(1, 3, 5)

A Irwin(11) investigated the suitability of 57 species of freshwater fishes for this purpose. Briefly, they should be of local importance, they must be a type that can be maintained in good health in the laboratory in the dilution water to be used, and enough must be employed so that reasonable statistical reliability is assured (for example, 10 per tank).

1 Fish are usually employed as test animals, although there is no reason not to use any other organisms that can be successfully kept alive in the test tanks.(12)

2 Continuous availability is also important. It is well not to change the test species after a program has been established, since the reactions of different species to the waste being monitored may not be the same.(11)

3 Animals in the exposure tanks should be fed the same as those in the stock tanks. The same kind of food in the same ratio of food to weight of test fish should be added to each tank at the same intervals. Unnatural acceptance of food in exposure tank may indicate a measure of distress, even in the absence of mortality.

B Oxygen determinations should be run occasionally to ensure that any deleterious symptoms are the result of the effluent and not of oxygen deficiency. Actual minimum acceptable levels will depend on the temperature and type of fish used.

C Long-continued exposure to low level concentrations in tanks may result in cumulative intoxication, or acclimatization.

1 In most cases these effects can probably be best counteracted by periodic renewal of the test fishes, for example: at 60-day intervals.

2 When obtaining stocks of test animals from receiving waters, these same factors should be borne in mind. Fish or other organisms taken from below the outfall might have acquired some immunity or sensitivity to the effluent being tested.

3 Fish or a species normally present in the receiving stream, but imported from some other (unpolluted) source, will presumably exhibit a completely unconditioned response. Generally, since the aquatic life to be protected is that already present in the stream, the most logical source of fish is the stream itself. Under operating conditions, however, it is not always practicable to collect the experimental animals from this source and imports from another area may be necessary.

D Selection of Dilutions

1 The "critical range" of toxicity may be defined as the range between the highest concentration that kills no test animals and the lowest concentration that kills all. The TL_m of the conventional bioassay(3) is in the middle portion of this range.

2 When an effluent is to be biomonitored under Plan B, the selection of appropriate dilutions might be based on the above concept of "critical range."

  a If a "tight" control is desired, the highest concentration might be established near the TL_m. When a batch of test animals is first placed in such a dilution, approximately half of them may (by design) be expected to die. The survivors, however, would constitute a rigorous control as any increase in toxicity would be expected to kill the remaining animals in order of susceptibility, until, as the top of the critical range is reached, all would be dead.

  b A somewhat less stringent control would be effected with a dilution near the lower end of the critical range.

3 In any large population of test animals kept in an exposure tank over an extended period of time, an occasional animal may be expected to die.

  a The mortality of significance then is not the occasional individual death, but the sudden death of 25, 50, or 100% of the test animals.

  b When this happens, biomonitoring has sounded the alarm to take appropriate action to detoxify the effluent or to divert it from the receiving stream until it is again normal.

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THE STATISTICS OF TOXICITY TESTS

I EXPERIMENTAL DESIGN

In general terms a toxicity test is used to investigate the effect of a toxic agent on a test organism. Because of biological variation between individuals, in order for the test to be meaningful, the effect must be studied for a group of individual test organisms, not just one individual. In addition, an investigator generally runs a toxicity test to determine the level of a toxic agent that will produce a given degree of effect, i.e., to determine an endpoint. Therefore, in practice, a toxicity test is usually run to determine the level of a toxic agent that produces a defined endpoint in a population of test organisms. Usually this is accomplished by exposing portions of the population to different levels of the toxic agent and observing the effect of the toxic agent on the various portions. In the Acute Mortality Test, this means observing the percent that is killed at each level of the toxic agent and then determining the level that would kill 50 percent of the population. For a toxicant, this is called the median lethal concentration (LC50). This test procedure imposes certain requirements on the experimental design of the test if the results are to valid.

A Randomization

The first requirement is randomization, both of the test animals and the test chambers. Randomization of the test animals is important so that the portion of the population exposed to each level of the toxic agent is representative of the whole population--at least as representative as one can make it. Randomization of the test chambers is important to minimize the effects of external factors on the results of the test. There are several ways randomization can be performed, such as by drawing cards out of a hat or using a table of random numbers. In general, stratified randomization is better than total randomization.

B Replication

The second requirement for a good toxicity test is replication of test chambers. Duplication is about as far as most investigators will go. Replication is needed because randomization cannot overcome all problems. One must get an idea how much variation exists in the test, and the only way to do this is through replication. There are two kinds of replicates--those run at the same time and those run at different time. Sometimes it is said that replicates run at the same time measure reproducibility, and those run at different times measure repeatibility.

C Numbers of Subjects.

A third requirement is to have an adequate number of test animals in the population and in the portions of the population. This is replication of test animals. Five animals per portion is about a bare minimum; ten is a good compromise between theoretical and practical necessity. It is obvious that if only five animals are used in a portion, the results for a portion can only be 0, 20, 40, 60, 80, and 100 percent. A difference of one animal between duplicate portions means a difference of 20 percent. If there are ten animals per portion, a difference of one animal will mean only a 10 percent difference. Even the best random distribution cannot make the replicates identical, so there must be enough animals in each replicate to minimize the consequences of such differences.

D Number of Partial Kills

(1) A fourth requirement of a good experimental design for toxicity tests is that there be enough levels of the toxic agent tested so that the level producing 50 percent kill can be determined accurately. If one only tests levels that kill either 0 percent or 100 percent of the animals, all he knows is that the LC50 is between two of the levels tested.

If, on the other hand, the investigator tests levels that kill 0, 20, 40, 60, 80, and 100 percent of the animals, he can determine the LC50 rather accurately. There are biological and practical limitations on how accurately one can determine an LC50. However, if results are to be very meaningful, as a bare minimum the test should have two concentrations that produce partial kills. With two partial kills, one can calculate the LC50 and its confidence limits with fair accuracy.

(2) The degree to which an investigator worries about each of these requirements must depend on the confidence one wants to have in the data. One can ignore all four requirements and still get a "ball park" figure, but "ball park" results are not very useful. Of course, it is ridiculous to go into great detail on the statistical requirements for a good toxicity test and ignore other things such as the biological and chemical requirements.

II ANALYSIS OF THE DATA

A Once the test has been run, the remaining problem is to calculate the results from the data collected. However, what can be done with the data is often limited by what data were collected and how they were collected. Thus the design of the experiment should take into account the ultimate use planned for the data. The only data collected from most routine toxicity tests is concentration-mortality data.

B There are several methods for analyzing concentration-mortality data. It is generally accepted that the best way is to determine the median lethal concentration (LC50). Statistically this is a good endpoint and it is about as useful as any other one. All of the methods for calculating an LC50 can be visualized as graphical methods based on a plot of concentration vs. percent mortality. What changes from method to method are the coordinates and the means of connecting the points. Standard Methods describes the commonest graphical method, but in general graphical methods are approximate methods, give no measure of confidence limits, and are not useful with certain kinds of data. Most people who want to use a better method use the Litchfield-Wilcoxon method. This is a semi-graphical approximate probit method, but this is rather time consuming unless a computer can be used. Others use various other parametric or nonparametric methods, such as the logistic method of Bergson or a moving average method. All of these are discussed by Finney. Some of these methods assume a particular relationship between concentration and mortality and some do not. Generally it is impractical to try to get enough data to prove whether or not a specific relationship exists. Fortunately, most often the calculated LC50 and its confidence limits are about the same for all computational methods.

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THE SELECTION OF ENDPOINTS FOR TOXICITY TESTS

I INTRODUCTION

An endpoint in a toxicity test is a defined magnitude of a specific observed effect of a toxic agent on a living organism. Thus the selection of an endpoint involves both the selection of the effect and the selection of the magnitude. At one time "toxic" was generally used to mean "to cause death" and death was almost the only effect studied. However, today it is generally recognized that toxic agents cause many damaging effects other than death, and so "toxic" is used to mean "to cause an adverse effect" and "lethal" is used to mean "to cause death."

II DEATH

A Death was probably the first effect used in toxicity tests and is still the most widely used effect because it possesses four very useful properties:

(1) It applies equally well to all organisms;

(2) It applies equally well to all toxic agents;

(3) Usually it can be detected rather easily without the use of specialized equipment;

(4) It is an obviously important adverse effect.

Because of these properties, death will probably always be the basic observed effect for toxicological studies.

B If an endpoint is to be defined using death as the effect, in terms of a group of subjects, there are several magnitudes of death that can be used, such as:

(1) the lowest concentration that kills all of the subjects (LC100)

(2) the concentration that kills 50% of the subjects (LC50)

(3) the highest concentration that kills none of the subjects (LC0).

C It has been found that statistically and practically the LC50 is the best endpoint. Although it is sometimes argued that LC0 should be a more useful endpoint, the LC0 is difficult to determine.

III OTHER EFFECTS

There are many effects other than death that can be and have been used. Warner (1967) reviewed many histological, physiological, biochemical, behavioral, activity and growth effects and endpoints. The possibilities are only limited by man's ingenuity, time, and money.

IV CRITERIA FOR THE SELECTION OF ENDPOINTS

A From all the effects that can possibly be used for toxicity tests, one must choose the best effect for one's own tests. Death is obviously widely used effect, but it is generally not sensitive enough. From the more sensitive effects one must choose one that is practical and will meet the needs of the experiment.

B Generally the first consideration is time and equipment, and these are obviously important, but usefulness should be the primary concern. Water pollution control is a matter of solving practical problems. Thus an effect should be useful. For many of the effect that can be used in short, sensitive tests, there is no information on usefulness and some of these require elaborate equipment.

V CHRONIC TESTS

Much of the work of the National Water Quality Laboratory is now centered around what we call the chronic test. In these tests the animals are exposed to the toxic agent before and during spawning and the eggs and fry produced are exposed for about thirty days or more. In these tests, we look for effects on survival, growth and reproduction, because such effects are obviously important and are rather easy to study. Chronic tests generally last for eleven months or more. In addition, we are studying the usefulness of some other sublethal effects by comparing the results of acute tests with those of the chronic tests.

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ALGAL GROWTH POTENTIAL TEST

I INTRODUCTION

Dense growths of algae in surface waters are aesthetically undesirable, cause problems in water treatment, produce changes in the aquatic environment that are harmful to fish and other aquatic life, and are symptomatic of pollution. The density of phytoplankton populations is directly related to the concentration of nutrients. This relationship has been well documented, and is now embodied in the concept of trophic level or trophic status of surface waters. One or more of the following parameters are commonly used to describe the trophic status: (a) nutrient concentration - principally N and P, (b) algal count, (c) chlorophyll concentration, (d) primary productivity, (e) particulate organic matter, (f) oxygen depletion in the hypolimnion, and (g) phytoplankton species composition or indicator species (Rawson 1956; Davis 1964; Goldman & Carter 1965; Oglesby & Edmondson 1966; Fruh, Steward, Lee & Rohlich 1966).

II EUTROPHICATION

Three general trophic levels now recognized, here arranged in ascending order, are: oligotrophic (low), mesotrophic (intermediate), and eutrophic (high). The addition of nutrients to surface waters raises the trophic level and results in an increase in phytoplankton density and changes in the species composition. This process, commonly referred to as eutrophication, is greatly accelerated by the discharge of nutrient-laden domestic and industrial wastes (Hasler 1947), Edmondson & Anderson 1956).

III MEASUREMENTS OF TROPHIC LEVELS

Although chemical analyses provide information on the concentration of nutrients, their availability to the algae can be determined only by biological assay. Biological assays to determine the potential (algal) productivity of surface water were first used in the late twenties (Schreiber 1927) and early thirties (Strom 1933), but until recently had been used only infrequently (Potash 1956; Skulberg 1964, 1967; Shelef & Halperin 1970). In 1967, the Joint Industry-Government Task Force on Eutrophication took steps to develop a standardized algal growth potential (AGP) test. Using this test, one can:

A Evaluate the effectiveness of waste treatment processes in removing elements that support or stimulate the growth of algae.

B Determine at what point along the time scale of progressing eutrophication the water of a given lake or stream happens to lie (trophic status).

C Anticipate the effect on algal production of introducing extraneous nutrients.

D Determine the extent to which nutrient levels must be reduced in a body of water to effect an acceptable remedy.

IV BASIC STEPS OF ALGAL GROWTH POTENTIAL TEST

A A surface (test) water sample is collected and the indigenous microorganisms are removed by filtration (0.45 micron membrane filter at 15 inches of mercury) or ultracentrifugation.

B The surface water and standard medium (Table 1) are inoculated with 1000 cells/ml of *Selenastrum capricornutum*, or 50,000 cells/ml of *Anabaena flos-aquae* or *Microcystis aeruginosa*.

C The cultures are prepared in triplicate and incubated 7-10 days at 24°C, 200 fc (blue-greens) or 400 fc (*Selenastrum*) continuous illumination, with shaking at 100 oscillations/min (culturing may be by flask, chemostat, or *in situ* technique).

D Algal growth is measured daily by (1) cell counts, (2) determining the

TABLE 1

MAY, 1970 VERSION OF PAAP NUTRIENT BASAL MEDIUM

(This formula consists of 30% of the concentrations of the macroelements listed in the February, 1969, PAAP Booklet. The Na_2CO_3 was replaced by $NaHCO_3$, and the EDTA was reduced to 333 μg/l.)

MACROELEMENTS: (milligrams per liter)

Compound	Final Conc.	Element Furnished	Element Conc.
$NaNO_3$	25.500	N	4.200
K_2HPO_4	1.044	P	0.186
		K	0.469
$MgCl_2$	5.700	Mg	1.456
$MgSO_4 \cdot 7H_2O$	14.700	Mg	1.450
		S	1.911
$CaCl_2 \cdot 2H_2O$	4.410	Ca	1.202
$NaHCO_3$	15.000	Na	11.001

If the medium is to be filtered, add the following trace-element-iron-EDTA solution from a single combination stock solution after filtration. With no filtration, K_2HPO_4 should be added last to avoid iron precipitation. Stock solutions of individual salts may be made up in 1000 ×'s final conc. or less.

MICROELEMENTS: (micrograms per liter)

H_3BO_3	185.5	B	32.5
$MnCl_2$	264.3	Mn	115.4
$ZnCl_2$	32.7	Zn	15.7
$CoCl_2$	0.780	Co	0.354
$CuCl_2$	0.009	Cu	0.004
$Na_2MoO_4 \cdot 2H_2O$	7.26	Mo	2.88
$FeCl_3$	96.0	Fe	33.05
$Na_2EDTA \cdot 2H_2O$	300.0		

chlorophyll content, in vivo fluorescence, light scattering or optical density (600 nm) of the culture, (3) measuring the C-14 uptake, or (4) determining the dry weight of the algae at the end of the incubation period. Regardless of the parameter used to measure growth response, the result should always be expressed in terms of the final dry weight of the culture.

E The growth response of the alga in the test water is compared to its growth in the standard medium.

V PHASES OF THE TEST STILL UNDER STUDY INCLUDE:

A Composition of the standard growth medium.

B Effects of ventilation and shaking on the growth response of batch cultures.

C Techniques of measuring growth response.

D Techniques of removing indigenous microorganisms from test surface waters.

VI For copies of the Provisional Algal Assay Procedure and information on the availability of subcultures of the test organism, contact:

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EFFECTS OF POLLUTION ON FISH

I INTRODUCTION

A By what means do pollutants exert their effects?

B What is the relationship between water quality and water use by fishes?

C What is the reaction of fishes to domestic sewage?

D Is there any noticeable change in species composition of the population following pollution?

E Is there any genetic or environmental selection in favor of pollution resistant strains?

F Nearly any pollutant, given sufficient concentration and time, can kill as a direct toxicant. We are primarily concerned here with sub-lethal or chronic levels of pollutants. (Acute toxic levels and physiological mechanisms are treated elsewhere.)

II MECHANISMS OF DETRIMENTAL ACTION

A Inert silt may

1 Clog gills and smother eggs and fry,

2 Blind sight feeders and eliminate hiding places,

3 Smother food organisms,

4 Reduce oxygenation by smothering algae.

B Irritants may

1 Act as repellents,

2 Cause excessive mucous secretion and upset osmotic balance.

C Sub-lethal quantities of a host of environmental materials are constantly penetrating the bodies of fishes by various routes. We are generally not aware of their presence unless they:

1 Cause an observable effect on the fish,

2 Cause an effect on man by imparting off-taste or odor to fish flesh,

3 Are sought for and detected, e.g.; radioactive substances, DDT, mercury.

We can only speculate as to their undetected effects.

III ENVIRONMENTAL RELATIONSHIPS BETWEEN WATER QUALITY AND WATER USE BY FISHES

A Freshwater fishes sometimes spend their entire lives in a single body of water. Pollution of that body of water therefore impinges on them at every stage of their life cycle, and at every point in their various ecological relationships; such as, seeking food or escaping enemies.

B Migratory fishes on the other hand feed and grow up in one body of water (the ocean for anadromous species, fresh water for the catadronous eels), then travel a migration route (usually a river) to another body of water where they breed.

Pollution at either end of the route, or a pollution block along the migration route, may eliminate the species.

C What will affect one species adversely may be favorable for another.

1 Cold water species, such as various trouts, might be killed or eliminated by warmed water from a power plant which would in turn permit the survival of warm water species, such as certain basses, sunfishes, etc.

2 Benthic species (such as catfish, sculpins, or suckers, which live near the bottom) might be eliminated by a smothering

blanket of inert material which would not affect limnetic species inhabiting the open water areas (such as white and yellow bass, gizzard shad, or walleye). The limnetic species on the other hand might be inhibited by a dense turbidity which would hide their prey, suppress the growth of nutritious plankton, or clog their gills; this in turn being relatively harmless to the benthic group.

3 Likewise the shoreline hugging littoral forms (like pumpkinseed or bluegills) and profundal species (such as lake trout) might respond selectively to such factors as temperature or transparency.

IV RESPONSE OF FISHES TO SEWAGE AND SIMILAR WASTES

A These wastes in general are not toxic in themselves, but exert their effects on fishes directly.

B Oxygen Depletion

1 May lead to death at various stages in life history depending on circumstances.

2 May lower resistance to disease or increase sensitivity to intoxication.

3 May reduce ability to capture food or swim against current.

C May smother or kill normal food sources.

D May increase normal fish production through eutrophication.

E Usually changes normal population balance by driving out predatory types and encouraging scavengers.

F Reported to cause osteological and other pathological manifestations, such as the knothead condition of carps in the Illinois River.

V NATURAL SELECTION AND ACCLIMATIZATION TO POLLUTION

A Known biological mechanisms for selective breeding of pollution resistant strains operate in nature among fishes as among other organisms.

1 Studies of population genetics indicate that after some finite number of generations of population stress (e.g.; exposure to a given pollutant), permanent heritable resistance may be expected to develop.

2 If the environmental stress (or pollutant) is removed prior to the time that permanent resistance is developed in the population, reversion to the non-resistant condition may occur within a relatively few generations.

3 Habitats harboring populations under stress in this manner are often marked with the dead bodies of the unsuccessful individuals.

B Individual organisms on the other hand can over a period of time (less than one life cycle) develop a limited ability to tolerate different conditions, e.g.; pollutants:

1 With reference to all categories of pollutants both relatively facultative and obligate species are encountered (e.g.; euryhaline vs. stenohaline, eurythermal vs. stenothermal).

2 This temporary somatic acclimatization is not heritable.

C A given single-species collection or sample of living fishes may therefore represent one or more types of pollution resistance:

1 A sample of an original population which has been acclimated to a given stress *in toto.*

2 A sample of the surviving portion of an original population, which has been "selected" by the ability to endure the stress. The dead fish in a partial fish kill are that portion of the original population unable to endure the stress.

3 A sample of a sub-population of the original species in question which has *in toto* over a period of several generations developed a heritable stress resistance.

D Any given multi-species field collection will normally contain species illustrative of one or more of the conditions outlined above.

VI POPULATION COMPOSITION RESPONSES TO POLLUTION

A Sewage pollution generally results in a reduction in the predatory types and their replacement by scavengers. Regions of severe oxygen depletion may be devoid of fish, or inhabited only by rough fish such as gar or carp. The general concept of a reduction of variety coupled with an increase in abundance in certain regions is as valid for fishes as for other groups.

B Population responses to toxic pollution are unpredictable except that reduction in variety is again almost sure to result.

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APPLICATION OF RESULTS OF THE TOXICITY BIOASSAY

I INTRODUCTION

A The TL_m is an estimate of a midpoint.

B It is thus not usable directly as a dilution factor.

C From this relatively precise value, we must extrapolate to a "safe concentration" in the receiving water.

II Extrapolation is accomplished by means of the "application factor". This is a multiplier used to reduce the TL_m value to a magnitude estimated to be relatively harmless to aquatic life in the receiving water.

A Definitive values for the application factor under various circumstances must be established by investigation.

B The development of application factor is complicated by many circumstances including the following.

1 The toxicity bioassay is designed as a laboratory method. In its basic form it can only evaluate one batch of toxic material against one batch of receiving water.

2 The concentration of toxicant in a static test containers may be reduced during the period of the test by the loss of volatile materials, precipitation or complexing with materials in the test water, adsorption or precipitation with mucous, etc. (By contrast with field conditions where solutions at any one point are constantly renewed).

3 In operative applications, both wastes and receiving waters may change.

a Wastes may vary in composition, quantity, or both.

b Receiving waters are subject to quantitative, chemical and physical changes resulting from season or weather, and changes resulting from variations in upstream pollution load.

c Synergistic or antagonistic effects may result from the above changes.

4 Other species of fish, or other life history stages of the same species might respond differently.

5 Fish food organisms and other stream inhabitants might respond differently.

6 Toxicities frequently increase with temperature, low dissolved oxygen, or other adverse environmental changes.

7 Chronic or cumulative toxicity, i.e., effects on growth rate, reproductive capacity, or other attributes essential to survival, may not be discernible for weeks or months.

a The static jar test is designed to test "acute" toxicity only.

b Comparison of successive TL_m's provides a basis for estimating the effects of long-term exposure. This is easily done graphically.

c Special procedures are being developed in an effort to extend the bioassay test over a longer period of time.

1) Periodic renewal of solution is useful.

2) Continuous flow apparatus has been developed to the point where it is now a practicable tool for both research and for routine operations.

d Conclusive evidence that pollution has been entirely harmless to stream life after an extended period of time can be obtained only by careful and protracted study of the receiving water.

C The Application Factor

The combined effects of the various factors mentioned above is, at the present time, largely unpredictable, empiracle observations are consequently our prime recourse.

1 In the absence of specific evidence to the contrary, if circumstances demand the citation of an application factor regardless, the value 10 (or 1/10th of the TL_m) is suggested as a starting point. This value will be used in the examples cited below.

2 It is anticipated that in time, special application factors will be developed for various types of wastes under different circumstances. Examples of the considered use of smaller factors can be cited.

3 No generally acceptable formula to derive application factors has yet been developed.

III COMPUTATION OF A RATE EFFLUENT DISCHARGE WHICH WILL BE SAFE FOR STREAM LIFE

A Perform bioassays to obtain TL_m values.

B Compute the total volume (or flow) of water required to dilute the effluent to the TL_m concentration. This is the "dilution volume".

$$\text{Dilution Volume (DV)} = \frac{100 - TL_m \text{ (in percent)}}{TL_m} \times \text{Effluent Flow}$$

C Multiply the Dilution Volume (in percent) by the application factor F_a to give the total flow requirement for safety to aquatic life Q_s.

$$Q_s = DV \times F_a$$

If actual minimum stream flows are greater than this value, no problems need be anticipated, if less, "something must give".

Obviously the two above manipulations could be readily combined, but the easily derived "dilution volume" can be put to certain special uses as is mentioned elsewhere.

Example 1:

Given $TL_m = 5\%$

Effluent flow $(Q_e) = 10$ cfs

Application Factor $F_a = 10$

$$DV = \frac{100 - 5}{5} \times Q_e$$

$$DV = 19 \times 10 \text{ (cfs)} = 190 \text{ cfs}$$

$$Q_s = DV \times F_a = 190 \times 10 = 1900 \text{ cfs}$$

Does the receiving stream have a minimum flow of 1900 cfs or greater?

Example 2:

Given $TL_m = 5$ mg/liter, or .0005%

$Q_e = 10$ cfs

$F_a = 10$

$$DV = \frac{100 - .0005}{.0005} \times Q_e$$

$DV = 199,999 \times 10 = 1,999,990$ cfs

$Q_s = DV \times F_a$

$Q_s = 1,999,990 \times 10 = 19,999,900$ cfs

Does the receiving flow have a minimum flow of nearly 20 million cfs? If not, either:

1 The TL_m (toxicity) of the effluent must be reduced (by treatment).

2 The effluent volume must be reduced.

3 The river flow must be increased, or damage to aquatic life may possibly result.

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SPECIAL APPLICATIONS AND PROCEDURES FOR BIOASSAY

I INTRODUCTION

A The report of the Council on Environmental Quality (1970) repeatedly stresses the need for the development of predictive, simulative, and managerial capabilities to combat air and water pollution. The last capability depends on the first two.

B The standard static jar fish bioassay, which uses death as a response, enables one to predict the toxicity of a particular waste to fish. One limitation of this procedure is that it uses a grab sample which represents the quality of the waste at only one point in time. The water used to make the dilutions is also taken at one point in time. At the actual industrial site, the quality of the waste and the river water vary through time. A composite waste sample partially overcomes this limitation, but may mask variations that are biologically important.

C One could put fish in a continuous flow of waste diluted with river water, but then there is one further limitation of the standard bioassay: death is used as the response. In order to prevent damage to organisms, it is necessary to have an early warning of dangerous conditions, so that corrective action can be taken. In other words, symptoms of ill health, which occur before death, must be detected if there is to be time for diagnosis and treatment.

II METHODS AND MATERIALS

A Fish Movement Patterns

1 Fish movement patterns can be monitored using the technique of light beam interruption described in detail by Cairns, et al. (1970). Dawn and dusk are simulated by a motor-driven dimming unit which gradually increases the intensity of the room lights over a half-hour period starting at 6:30 a.m. and gradually decreases the intensity to 0 over a half-hour period starting at 6:30 p.m. The cumulative movement of each of six bluegill sunfish, a single fish per tank, is recorded every hour throughout a test except during the simulated sunrise and sunset when an additional record is made on the half hour. Each day is divided into four intervals, first half day, second half day, first half night and second half night (Table I). Before any statistical analysis can be performed, recordings for day 1 must be completed. After the cumulative movement for day 1 is recorded, statistical analyses are performed after the completion of each designated time interval. For example, the cumulative movement recorded hourly for each fish during day 1, first half day values are compared to the cumulative movement recorded hourly for each fish during day 2, first half day values.

2 Based on the results of 20 laboratory experiments "stress detection" is defined as the presence of two or more abnormal movement patterns recorded during the same time interval.

B Fish Breathing

1 Breathing rates may be determined from polygraph recordings of breathing signals. The fish are tested in plexiglas tubes through which dechlorinated tap water or some toxic solution is metered at a flow rate of approximately 100 ml/min. Breathing signals are detected by three platinum wire electrodes placed in the water, an active electrode, an indifferent electrode, and a ground. The test chambers and methods of acclimating the fish are described in more detail by Cairns, et al. (1970) The photoperiod is the same as that for the fish movement study.

2 The fish are placed in test chambers by 6:00 p.m. and the recordings began at 6:00 a.m. the next day to allow the fish to recover overnight from handling. Toxic solutions are introduced at 10:00 a.m. after the experimental fish have been exposed to water containing no added

toxicant for periods of one to six days. Each experimental fish thus serves as its own control. In addition, one or two fish are never exposed to the toxicant and serve as controls throughout each experiment. In one experiment, using zinc as the toxicant, reported in Table VI, six control fish were exposed to water containing no added zinc for four days.

a Preliminary evidence suggested that the data could be analyzed by separating the experimental day into four periods; a period from 6:00 to 8:00 a.m. when the breathing rates changed markedly, a period from 9:00 a.m. to 5:00 p.m. when the rates were comparatively high, another period of rapid change from 6:00 to 8:00 p.m., and a night period from 9:00 p.m. to 5:00 a.m. when the rates were comparatively low (Sparks, et al., 1970).

b Bluegills increase their breathing rates when exposed to zinc (Cairns, et al., 1970). An individual fish was thus considered to have shown a response each time its breathing rate during a time period exceeded the maximum breathing rate observed during the corresponding period of the first day, before any zinc was added. A response was scored for each value on the second day that was higher than the first day maximum for the comparable period. The control periods (before any zinc was added) and the experiment where no zinc was added at all were used to determine how many false detections this method of analysis would produce. The experimental periods (after zinc was added) determined how quickly the method of analysis could detect zinc concentrations in water.

c Zinc concentrations were determined daily by atomic absorption spectrophotometry.

III RESULTS

A Fish Movement Patterns

1 Table 1 shows the results of one continuous flow experiment carried out for 20 days. During this experiment fish were exposed to zinc on day 7 from 1:00 p.m. until 7:00 p.m. at which time the flow was returned to normal dilution water. The zinc concentrations reached their maximum at 7:00 p.m. and atomic absorption analyses on effluent samples collected at this time showed the following concentrations: tank one, 13.32; tank two, less than 0.03; tank three, 11.39; tank four, 12.72; tank five, 13.32; and tank six, 12.59 mg/l Zn^{++}. The results show that these concentrations of zinc developing over the six hour interval of exposure were insufficient to cause a detectable change in the movement patterns of the fish. By 8:30 a.m. of day 8 the effluent zinc concentrations were less than 0.30 in all cases.

2 To determine the percent survival and recovery patterns of the fish once stress detection occurred, zinc flow was reinitiated at 1:00 p.m. on day 13 of this experiment. Between 8:00 and 9:00 p.m. on day 13 the zinc concentration in the effluent reached a maximum of: 7.51 for tank one; less than 0.05 for tank two; 7.49 for tank three; 7.52 for tank four; 7.49 for tank five; and 7.54 mg/l for tank six. The concentrations remained near the above values until the statistical analyses showed "stress detection" during the first half night values on day 14 (Table 1). As soon as stress detection occurred the flow was returned to normal dilution water. At 10:00 a.m. on day 15 zinc analyses showed all effluent concentrations to be less than 0.70 mg/l Zn^{++}. Stress detection continued to be registered for two consecutive time intervals following the initial detection, but after that no stress detection was registered and the frequency of abnormal patterns returned to prestress levels within 48 hours. In this experiment

as with all others in which dilution water containing zinc was replaced with dilution water minus zinc at that time of stress detection all fish survived!

3 The results from the series of experiments at progressively lower zinc concentrations indicate that the lowest detectable concentration is between 3.65 (Table II) and 2.93 mg/l zinc (Table III) for a 96-hour exposure.

B Fish Breathing

1 Table IV shows the breathing rates of five fish on days 1, 2, and 7 of experiment 8. The first four fish were exposed to a measured zinc concentration of 4.16 mg/l, beginning at 10 a.m. on day 7. The fifth fish served as a control and was not exposed to any added zinc. The amplitude of the breathing signals decreased every night, and the breathing rates for fish 2, in particular, could not be determined during some portions of the dark period (7:30 p.m. - 7:00 a.m.). The maximum breathing rates for each fish during each period of the first day are circled. The breathing rate of any fish during a time period of day 2 or day 7, which is greater than the maximum breathing rate recorded for that fish during the corresponding time period of the first day has a rectangle drawn around it. The total number of fish showing increased breathing is given at the bottom of each column. On day 2, fish 2 showed increased breathing on just two occasions. In contrast after zinc was added on day 7, three and four experimental fish at a time showed increased breathing.

2 Table V summarizes the results of successive comparisons of the first day maximal breathing rates to breathing rates on subsequent days (SCM method of analysis), for experiment 8. During the control period before any zinc was added there were 15 occasions when a single experimental fish responded, and three occasions when two experimental fish responded at the same time. At no time during the control period did more than two fish show responses together. After the zinc was introduced, all four of the exposed fish showed responses simultaneously on five occasions, and three fish showed responses during the same time interval on 19 occasions. If the criterion for detection of water conditions potentially harmful to fish were two or more responses during the same time period, then three false detections would have occurred before any zinc was added, and 4.16 mg/l zinc would have been correctly detected eight hours after it was introduced. If the detection criterion were three or more responses during the same time period, then no false detections would have occurred and the zinc would still have been correctly detected after eight hours.

3 The lowest zinc concentration tested was 2.55 mg/l. Using a detection criterion of simultaneous responses by three fish, this concentration was detected 52 hours after the zinc was added, with no false detections occurring during the four hours before zinc was added (Table VI). The responses of six control fish that were exposed to dilution water containing no added zinc are also shown for comparison. Note that there was no tendency toward increased breathing rates through time in the control fish, and that no more than one control fish showed an increased breathing rate during one time period.

4 Table VII summarizes information on three experiments that indicates the effectiveness of the SCM method of analysis when different criteria for detection are used. Changing the criterion for detection from one to three responses per time period generally increases the lag time and decreases the number of false detections. The lag time is the time from the addition of zinc to the first detection. A false detection is one occurring before any zinc is added to the water.

IV DISCUSSION

A The experiments described above show that the movements and breathing rates of bluegill sunfish can be used to detect sublethal concentrations of zinc. The criterion for detection is a certain number of fish showing an arbitrarily defined response in breathing rate or activity during one time period.

B In choosing a specific criterion for detection, the risk of not detecting stressful conditions soon enough must be weighed against the risk of false detections, and the choice would probably be determined by the nature of the pollutant. If a pollutant is easily detected by the biological monitoring system, is slow-acting, and if the toxic effects are reversible, then the criterion for detection might be responses by 3/4 of the test fish, to avoid the false detections that would necessitate expensive remedial action or a temporary shut-down. On the other hand, an industry that produces an effluent containing a fast-acting toxicant whose effects are irreversible would probably use a criterion that leads to rapid detection (responses by 1/4 to 1/2 of the test fish), and would have to go to the expense of installing holding ponds or recycling facilities to accommodate a relatively high number of false detections. Alternatively, a safety factor could be introduced by metering proportionally more waste into the dilution water delivered to the test fish than is delivered to the stream. The safety factor could be determined by growth and reproduction experiments with fish.

C In an actual industrial situation water and waste qualities are apt to vary unpredictably, and it would certainly be desirable to have a redundant detection system. It is conceivable that some harmful combination of environmental conditions and waste quality would be detected by monitoring one biological function, but not by monitoring another. It is also possible that excessive turbidity would disrupt the light beams of the movement monitor, and not affect the breathing monitor; or that an excessive concentration of electrolytes would affect the electrodes of the breathing monitor, but not affect the activity monitor. Therefore, the activity monitor and the breathing monitor have been combined in our laboratory for further experiments (Fig. 1).

D The rate of data acquisition and analysis could be greatly speeded up if the monitoring system were automated as shown in Figure 2. The sampling rate would be controlled by a minicomputer which could receive data from the movement monitor and the polygraph via a multiplexer as often as every minute. The minicomputer would be programmed to perform statistical analyses every 10 minutes, for example, and output the results on a teleprinter.

E Figure 3 shows how the fish monitoring units would be used at an actual industrial site. A monitoring unit would be located on each waste stream in the plant and on the combined waste stream. The experimental fish in each unit would be exposed to waste diluted with water from the river above the plant, and control fish would be exposed to upstream water alone (Fig. 4). The information from each monitoring unit could be analyzed by a central data processor, and when there was a warning response, the industry could tell which waste stream was at fault. If the problem was outside the plant, the control fish would show responses.

F Figure 5 shows how the in-plant monitoring systems would be integrated into a river management system. The in-plant monitoring units are shown as squares, and in addition to supplying information to each industry, the monitoring units also inform the control center. In such a system, there are several alternative damage prevention measures that could be used, in addition to whatever measures, such as shunting wastes to a holding pond or recycling wastes for further treatment, are available to each industry. If the monitoring units at Industry 2 indicate that toxic waste conditions are developing, then the control center might have Industry 1 hold its waste until the danger of combining wastes from Industry 1 and 2 in the river were alleviated by control measures at Industry 2. Alternatively, the control center might call for a release of water from the upstream dam to dilute the effluent from industry.

G It is likely that "fish sensors" in continuous monitoring units at industrial sites can warn of developing toxic conditions in time to forestall acute damage to the fish populations in streams. In conjunction with stream water quality standards for chronic exposure, such biological monitoring systems should make it possible for healthy fish populations to co-exist with industrial water use.

ACKNOWLEDGEMENT

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Descriptors:
Environmental Control, Bioassay, Toxicity, Fish Behavior, Fish, Monitoring

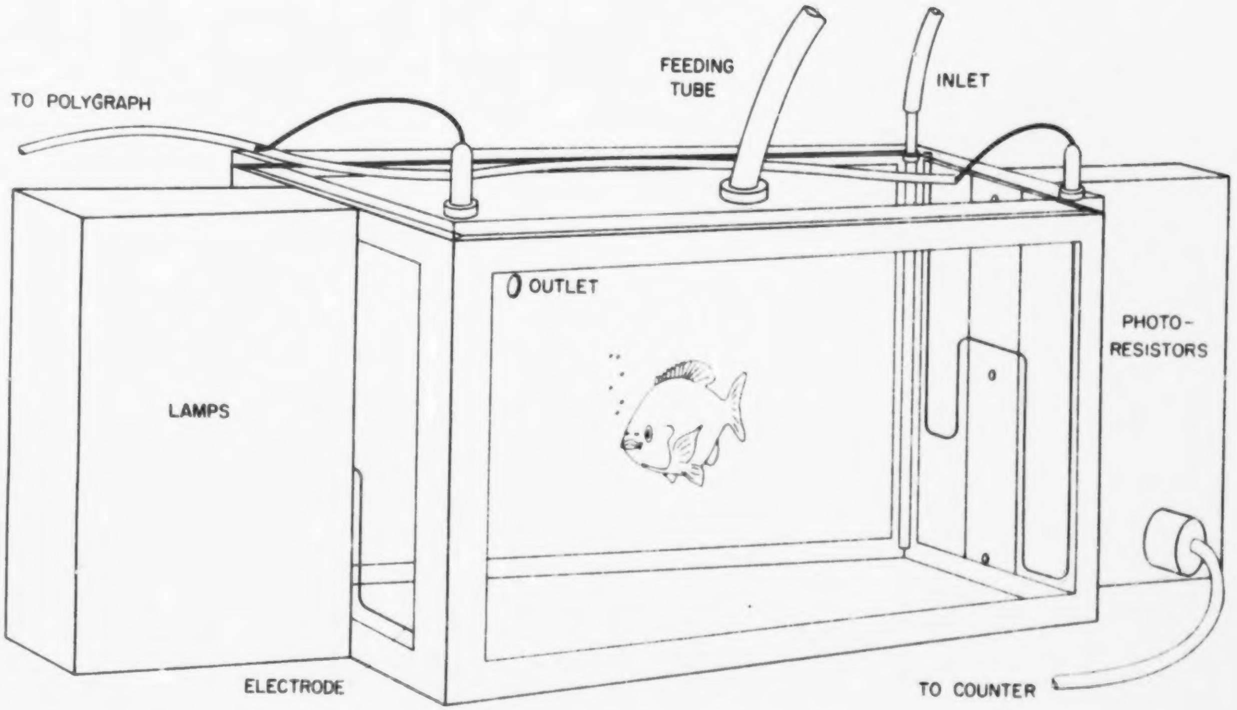
TO POLYGRAPH
FEEDING TUBE
INLET
OUTLET
PHOTO-RESISTORS
LAMPS
ELECTRODE
TO COUNTER

Figure 1. Test chamber for monitoring system, showing the electrodes for recording fish breathing and the light-beam system for recording fish movement.

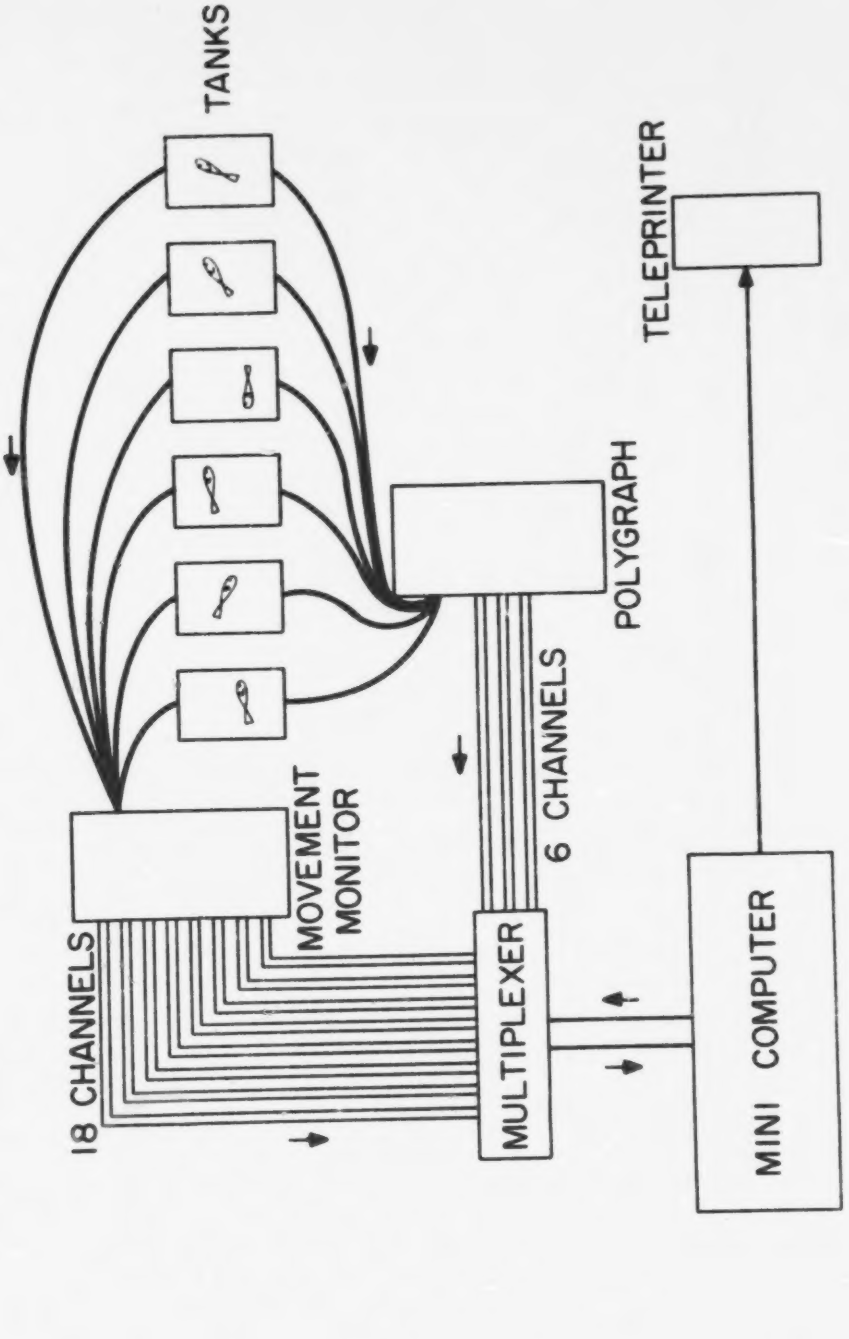
TANKS
TELEPRINTER
POLYGRAPH
6 CHANNELS
MOVEMENT MONITOR
18 CHANNELS
MULTIPLEXER
MINI COMPUTER

Figure 2. An automated fish monitoring unit.

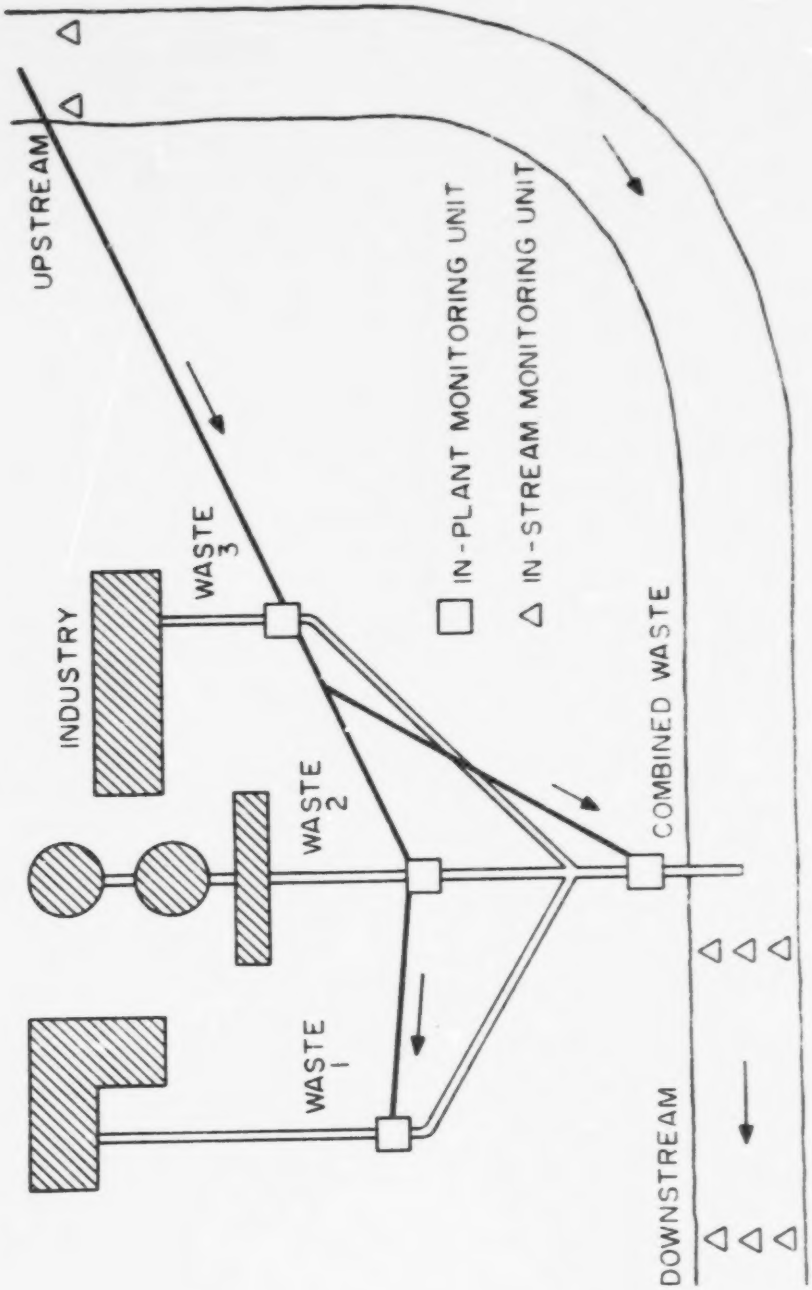
UPSTREAM
INDUSTRY
WASTE 3
WASTE 2
WASTE 1
COMBINED WASTE
DOWNSTREAM
□ IN-PLANT MONITORING UNIT
△ IN-STREAM MONITORING UNIT

Figure 3. Arrangement of fish monitoring units at an industrial site.

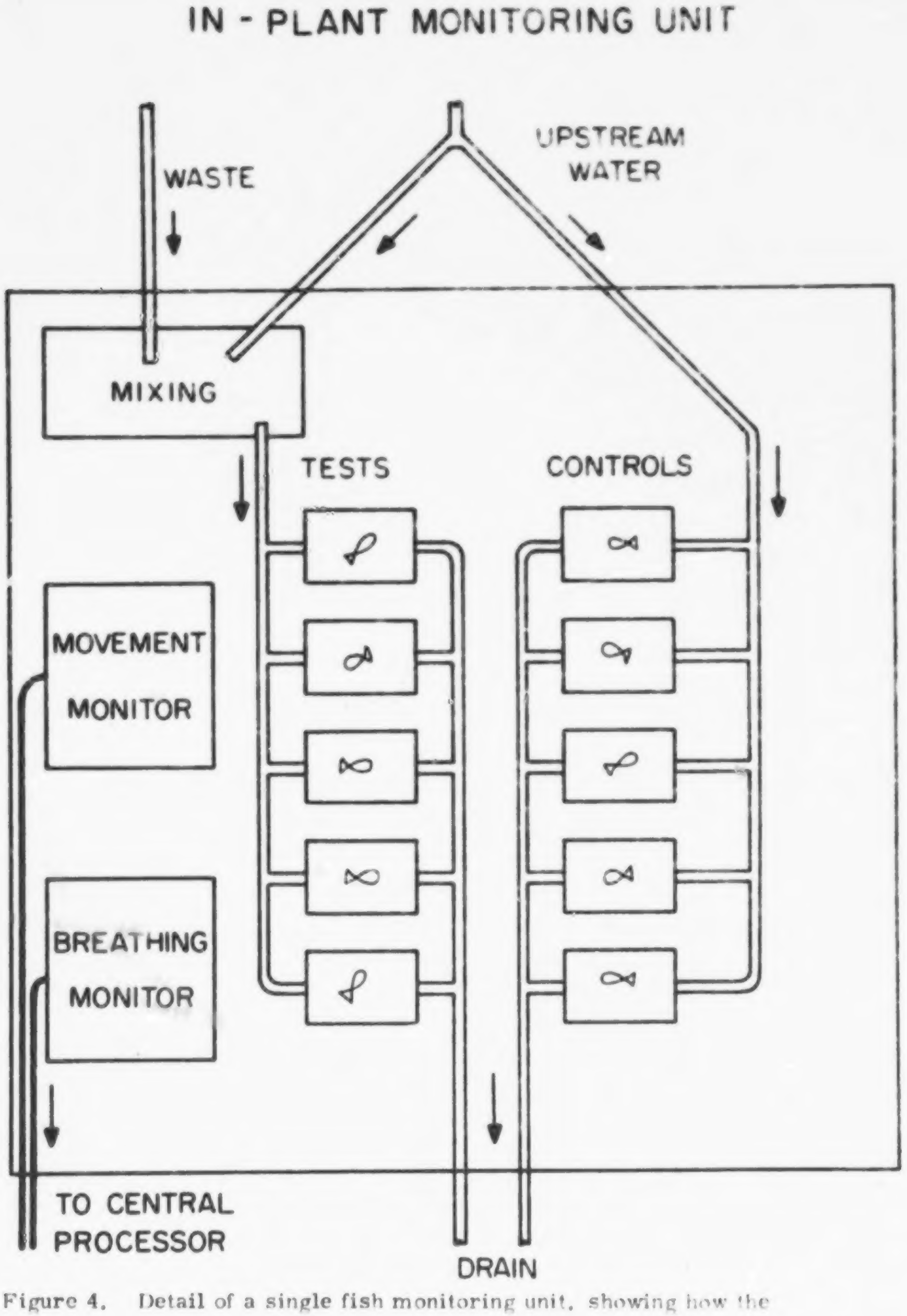
IN - PLANT MONITORING UNIT
WASTE
UPSTREAM WATER
MIXING
TESTS
CONTROLS
MOVEMENT MONITOR
BREATHING MONITOR
TO CENTRAL PROCESSOR
DRAIN

Figure 4. Detail of a single fish monitoring unit, showing how the experimental fish are exposed to waste diluted with upstream water and the control fish are exposed to upstream water alone.

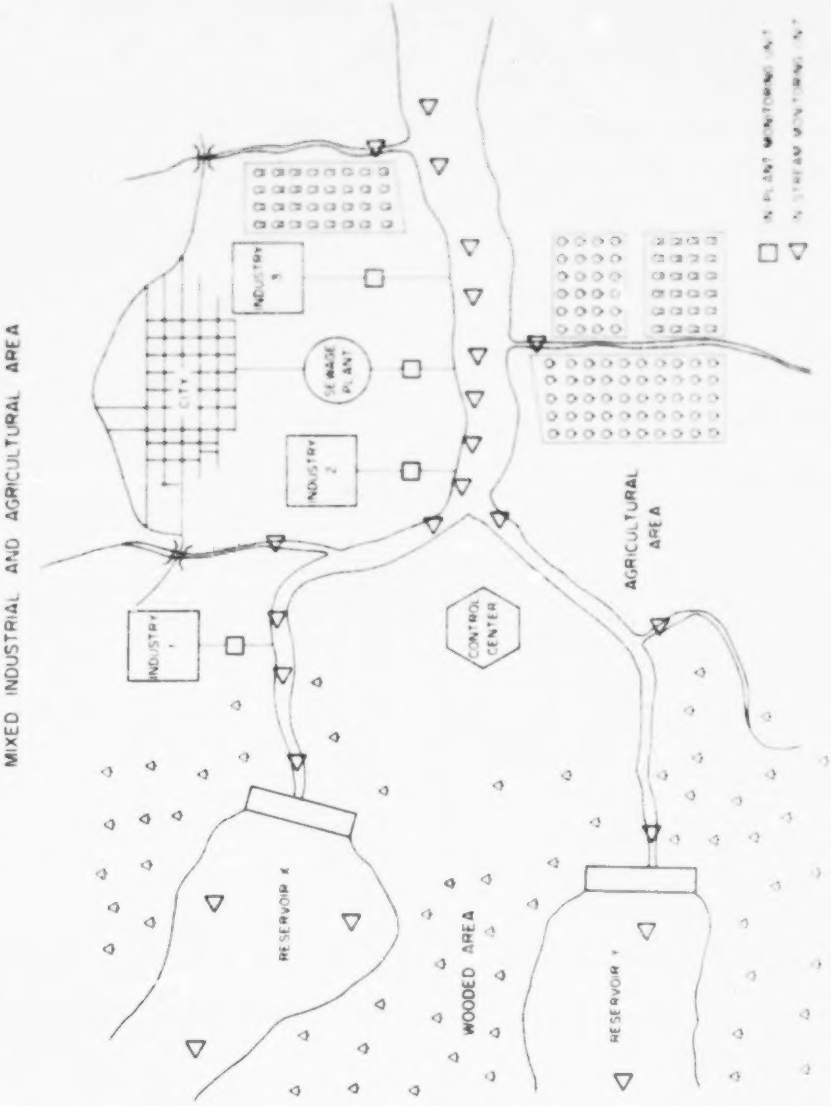
MIXED INDUSTRIAL AND AGRICULTURAL AREA
CITY
SEWAGE PLANT
INDUSTRY 3
INDUSTRY 2
INDUSTRY 1
CONTROL CENTER
AGRICULTURAL AREA
WOODED AREA
RESERVOIR X
RESERVOIR Y
□ IN PLANT MONITORING UNIT
▽ IN STREAM MONITORING UNIT

Figure 5. Use of in-plant monitoring units in a river management system

RIVER BASIN SURVEY PLANNING

I INTRODUCTION

A Ecologists must be willing to take a chance and predict the ecological consequences of alternative schemes for water resources management if we are to realize the maximum beneficial use of our resources. Historically, ecologists have lagged behind their engineering colleagues in developing prediction capabilities for a number of reasons.

1 Unfortunately, the operational characteristics of ecosystems are poorly understood when compared to the engineers systems. Engineers can predict that with so much concrete, steel, etc., and with a given amount of labor and money, a dam can be built on a river which will enable them to regulate flow behind the dam. The flow figures can be predicted with reasonable accuracy, and when the dam is built, the performance is generally within the original estimate. However, due to the complex chemical, physical and biotic interactions of an ecosystem prediction of the ecological consequences of any activity is more involved. The ecologist has developed only relatively recently rather primitive prediction systems for complex natural environments.

2 Another reason that ecologists have lagged behind engineers is that appropriate channels of information exchange between them have not always been open. Two contrasting philosophies have existed in the past which have hindered the ecological management of our water resources. Engineers, water resource economists and industrialists have generally had a construction philosophy of life which has conflicted with the conservationists or protective philosophy. Those who build power plants, dams, reservoirs, canals, etc., have used the technology of the time most economically appropriate with the expectation that what they are building will have a very short life span in terms of geologic time, and that when the structure is outmoded or uneconomical, it will be torn down or replaced. Plans are also strongly time dependent, and once completed must be initiated in a relatively short time period or they will become outdated. Complex sets of conditions involving technology, financing, land acquisition, power demands, etc., prevail; thus, the engineer is often characterized by a time and tide wait for no man attitude. The conservationist, on the other hand, realizes that once a rare species endemic to any area is lost, it is gone for all time, and that ecosystems once damaged may be difficult, if not impossible, to restore to their original condition.

B Fortunately, a new awareness on the parts of the advocates of both philosophies that our life support system on earth has two components, one industrial and the other ecological, has forced ecologists and engineers to work together. We currently realize that the survival of our present social system depends upon our ability to develop a harmonious relationship between these components.

C Those people involved in water resources management realize that some of the frustration and public outcry about water resources projects could have been avoided if proper consideration was given to ecological information before construction. Present trends in legislative action combined with the ever growing concern over the quality of the environment, the "environmental impact" of any new water resources development will be closely scrutinized. It is our objective here to briefly present some ecological information that should be considered along with other parameters in river basin planning.

II PRECONSTRUCTION ECOLOGICAL SURVEY

A One of the common problems of water resources development is to select a project site which allows maximum use without environmental degradation. In order to get this type of information, it is necessary to develop a series of prediction systems which will allow an ecologist to rank the potential construction sites. Perhaps one of the best ways to obtain important types of ecological information to be used in river basin planning is through a preconstruction ecological survey.

1 A preconstruction survey should be carried out by a team of chemists, ecologist, engineers, and taxonomists to get a complete picture of the chemical, physical and biological condition above and below the potential site location. If adequate background data are to be generated, the team should consist of one or more chemists, a bacteriologist, an algologist, a protozoologist, one or more invertebrate zoologists (including an aquatic entomologist), and ichthyologist, and a sanitary engineer. Since this involves a number of well-trained people, it can be moderately expensive.

2 The exact cost would depend on a number of factors including the size and structure of the river and the number of species likely to be encountered. Obviously, the lower Mississippi is a more difficult river to survey than a small river that one can throw a rock across. In addition, a stream already degraded by pollution is likely to have fewer species resulting in less cost for identifying the various organisms collected than an unpolluted stream with a very high number of species.

3 Before such a survey is contemplated, it is well to have a preliminary survey by a generalist used to dealing with these problems who can make a firm estimate of the costs involved and place reliable time estimates on completion of the project.

B A survey of this nature will provide a wide variety of information valuable in making a choice between prospective project locations.

1 It will establish a baseline of biological, chemical and physical water quality which can be useful in determining the waste assimilative capacity and other beneficial uses of the system. If one views the waste assimilative capacity of a river as a natural resource, then it is only logical to make use of that capacity along with other uses such as water supply, recreation and aesthetics to derive the maximum beneficial use from the system.

2 A preconstruction survey will determine pre-existing man made or natural stresses on the receiving system. In order to avoid blame, there is no better defense than an aggressive offense. Preconstruction data which documents the water quality is extremely valuable, particularly in receiving systems which are already partially under stress from other waste discharges. It is essential to establish the presence of natural stress on a system and thus avoid blame after project construction is completed and operations begin. Natural stress can take range from siltation and the introduction of organics from leaf litter, to thermal changes due to the introduction of underground aquifers.

3 How many water resources projects do you know that are located near critical spawning areas of striped bass, just above, or in the middle of an important fishery, in an area where the aquatic life is particularly vulnerable, and the like? Many of these situations could have been avoided through a preconstruction survey before site selection was made. Alterations in design of discharges could have received valuable input information based on this identification of valuable wildlife resources. For example, in some cases, it might be desirable to design waste discharge systems so that the waste is held against one bank of the stream or river leaving a free channel on the other side where migratory fish could pass through the area.

4 A preconstruction survey is a convincing demonstration that the resource developers are sincere in their efforts to protect the environment. The information derived from the survey can often furnish information about the ecological history of the area and make some predictions about future trends,

all of which are useful to engineers designing the waste disposal system and to the administrator concerned about public relations.

5 Through identification of critical physical, chemical and biological parameters, the preconstruction survey can help water quality personnel predict the mixing zone upon project operations. This is an important factor from a regulatory as well as public relations viewpoint.

III PREDICTIVE BIOASSAY

A Of equal importance to the preconstruction survey in project site selection is the availability of toxicity information based on a predictive bioassay. Even if a building site for an industry has been selected, but before construction begins or preferably before final designs of the plant are approved, some bioassays should be carried out.

1 These should be carried out with a simulated plant waste as close as possible in quality to the anticipated operating waste under the worst possible conditions.

2 Ideally, bioassays should contain three elements of the food chain, i.e., primary producers such as algae, invertebrates, and a fish. Just because a waste doesn't kill fish directly, it may ultimately prevent their survival due to its toxicity to an intermediate in the food chain. It is important to protect other elements of the aquatic community besides fish. For example, the loss of algae may impair the ability of the aquatic system to receive and transform organic wastes.

3 When conducting predictive bioassays, water quality of the proposed site location should be used since there are documented examples of synergistic interactions resulting in increased toxicity. If a plant is considering locating a system already receiving heavy metal discharges and plans to add additional heavy metals, then a predictive bioassay using the receiving water quality is essential since, for example, the toxicity of zinc combined with copper is ten times their individual toxicity.

B Predictive bioassays can be useful in helping determine a site selection for a variety of reasons. For example, an industry having a discharge containing heavy metals should consider locating in an area where the hardness of the water is high since for zinc the 96 hour TL50 in hard water is 10.1 - 12.5 ppm for the bluegill sunfish, where the 96 hour TL50 in soft water is 2.9 - 3.8 (McKee and Wolf, 1963).

C Predictive bioassays can be used to allow the plant to make maximum use of the system, to identify potentially hazardous interactions, and to see if preliminary waste treatment design is likely to be adequate, and if not, can be used to estimate the degree of additional treatment required.

D No longer can an industry be solely concerned with determining the acute toxicity of its waste products. Bioassays of industrial wastes have progressed from short-term tests using a single species with death as an end point to long-term tests involving several species and even communities of organisms. The use of respiration, growth, reproductive success, electrocardiogram movement patterns or other functional changes may replace the use of death as a criterion of response. The latter requires more time and expense but provides valuable predictive information concerning the "biologically safe concentrations" of various toxicants.

IV SIMULATION TECHNIQUES

A The development of simulation techniques involving the use of scale models becomes increasingly important as our population grows and more intensive use is made of the finite space available to us. In the past when we damaged an environment seriously, we could move on to a new undamaged environment and avoid most of the immediate consequences of poor management. Perhaps the last big movement of this sort in the United States was the exodus from the Dust Bowl. However, since most of the ecosystems of the United States are at or near tolerable stress levels, we no longer can go to virgin territory and escape our environmental mistakes. As a consequence, we can afford fewer mistakes without immediate penalty than we could in the past.

B One of the obvious protective measures we might take to prevent major ecological or environmental problems is to simulate prospective new uses in scale or laboratory models and restrict most of our mistakes to these. This practice is too common in engineering (for example, the U. S. Army Corps of Engineers river models at the Waterways Experiment Station, Vicksburg, Miss.) and industrial circles that it would hardly need mention were it not for the fact that ecological scale models or environmental simulation systems are not now commonly used.

C However, ecologists now are becoming quite interested in developing scale models to simulate various environmental systems and the practice should become increasingly common in the future. Of course, these suffer the weaknesses of all scale models and are still in primitive stages of development. They need not be extremely expensive and may be used to generate data which could be useful in preventing large scale mistakes. For example, many of the events which have occurred in Lake Erie could probably have been simulated in models.

D An example of a scale model we commonly use in our laboratory is a model stream to which we have attached a model steam condensing system allowing an incremental increase with a variable contact time in the condensor (Figure 1). Water from the model stream is passed through the condensor system and then through a series of plexiglass troughs where we allow algal and protozoan communities to establish. These experimental troughs are compared to control troughs, and some predictive information on the effects of passage through the system on downstream community structure determined.

V ECOLOGICAL QUALITY CONTROL TECHNIQUES

A Since we are a society almost compulsively dedicated to change, we are desperately in need of adequate prediction systems. The preconstruction survey, predictive bioassay and scale models previously discussed allow the ecologist ot make some of these predictions and help identify the various alternative uses which might be made of the environment and to estimate what the consequences of these will be. If these techniques were utilized in prospective water resource projects, we would be on our way to having adequate environmental planning. However, environmental planning alone will not be effective unless good quality control techniques are developed, as well as adequate environmental management practices.

B Just as in an industrial process where we have a system of checks and balances to insure product control, we must begin to develop the capabilities for environmental quality control provided an equitable environmental use plan can be developed.

1 This will require rapid biological, physical and chemical information systems, so that we get a continuous flow of information enabling us to predict unfavorable changes in our water resource systems.

2 Of the three types of information systems previously mentioned, the development of rapid biological monitors providing continuous information has lagged behind the other two in development. We can continuously monitor in a river many of the physical and chemical parameters

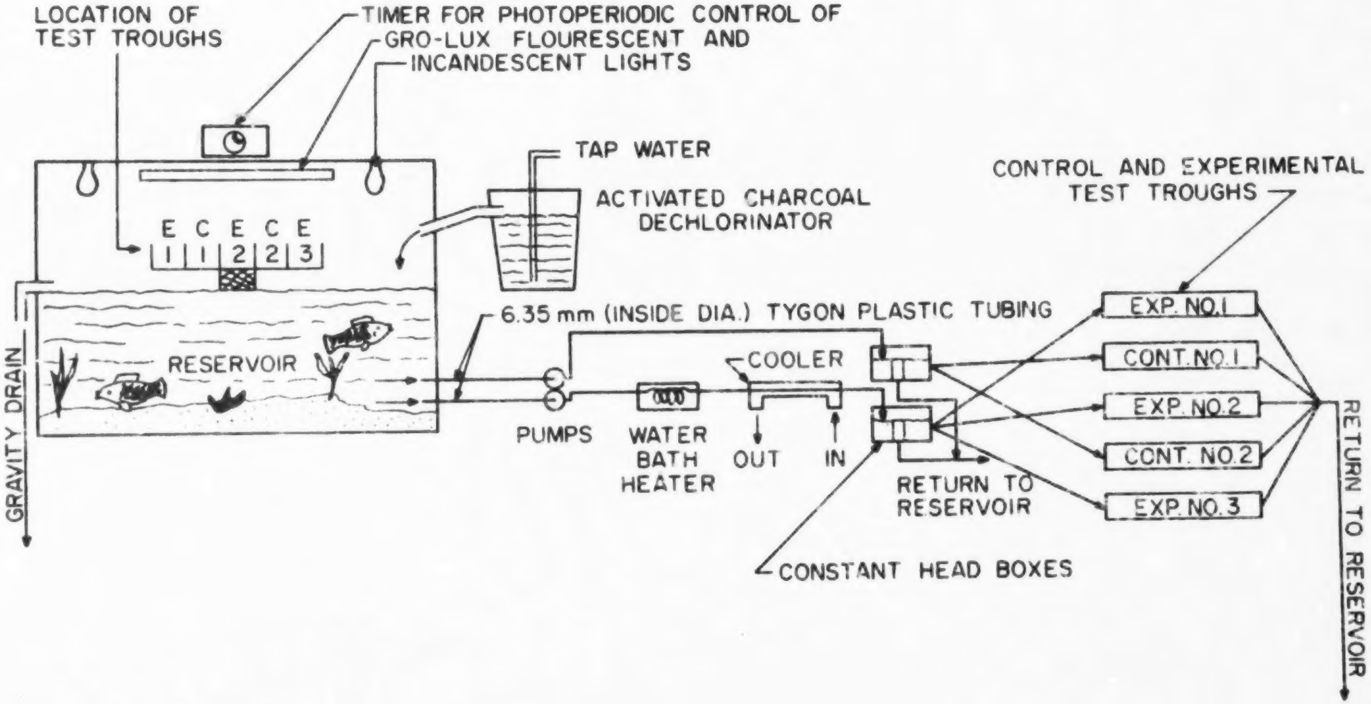
LOCATION OF TEST TROUGHS
TIMER FOR PHOTOPERIODIC CONTROL OF
GRO-LUX FLOURESCENT AND
INCANDESCENT LIGHTS
E C E C E
1 1 2 2 3
RESERVOIR
GRAVITY DRAIN
TAP WATER
ACTIVATED CHARCOAL DECHLORINATOR
6.35 mm (INSIDE DIA.) TYGON PLASTIC TUBING
PUMPS
WATER BATH HEATER
COOLER
OUT
IN
CONSTANT HEAD BOXES
RETURN TO RESERVOIR
CONTROL AND EXPERIMENTAL TEST TROUGHS
EXP. NO.1
CONT. NO.1
EXP. NO.2
CONT. NO.2
EXP. NO.3
RETURN TO RESERVOIR

FIGURE 1
SCALE MODEL SYSTEM FOR SIMULATING THE EFFECTS OF PASSAGE THROUGH A STEAM CONDENSOR ON MICROORGANISMS.

for analyses. However, the development of rapid biological information systems, both in-stream and in-plant, is essential to the maintenance of adequate environmental quality control.

3 We need to know the effects on biological organisms of a waste discharge before it enters the receiving stream as well as the biological effects after it enters the stream, and this information should be produced rapidly. Present systems are much too slow in view of the fact that the constituents of a waste stream are likely to vary from hour to hour and from day to day. Potentially disastrous materials should be detected before they enter a receiving stream if at all possible and at the very least, before substantial damage has been done in the receiving stream itself.

4 Several potentially useful methods for rapid in-plant monitoring are being explored (Cairns, et al. 1969) and one rapid in-stream method is now operational (Cairns, and Dickson, 1971). The in-plant methods just mentioned use changes in heart rate, breathing signal, and movements of the entire fish within a container to detect sublethal concentrations of toxicants in a waste discharge. If successful, these and other "early warning" in-plant systems could be used to determine the toxicity of a waste before it left the plant so that the appearance of a harmful concentration of a toxicant would activate a control system and shunt the waste immediately to a holding pond or recycle it for additional treatment.

C This continual information about the toxicity of a waste should enable sanitary engineers to identify periods of operation likely to produce the most toxic wastes, as well as identifying those components of the production process which contribute most of the toxicity.

D Full development of useful early warning systems with rapid information feedback will probably take a number of years and will require the close cooperation of a variety of disciplines. No doubt, the early developmental period will have its share of failures, but it is highly probable that effective systems can be produced and that their use will substantially improve environmental quality control. Since the ultimate test of the effectiveness of a waste treatment process should be in the receiving stream, in-stream early warning systems also should be developed to insure a continual flow of information.

VI SYSTEMS MANAGEMENT OF WATER RESOURCES

A Present advances in biological monitoring combined with physical and chemical monitoring capabilities indicate that in the near future we can develop and operate a river basin with varied water resource uses to maximize beneficial use without ecological damage.

B Figure 2 illustrates a river basin management system which includes reservoirs, agricultural uses, industries and towns, etc. Conceptually, utilizing a central control center and rapid physical, chemical and biological monitoring systems ecological damage could be prevented, through the operation of the system as a whole rather than each water resource user being concerned only with his own discharge.

C If an industry in the system had a spill of toxic material which was rapidly detected through the continuous monitoring systems, the following activities might be coordinated by the control center:

1 Upstream reservoirs could increase discharges for dilution of toxicants.

2 Municipal water users could curtail use of water and depend on reserves until toxicity was dissipated.

3 Downstream industries could shunt to holding ponds to prevent synergistic interactions.

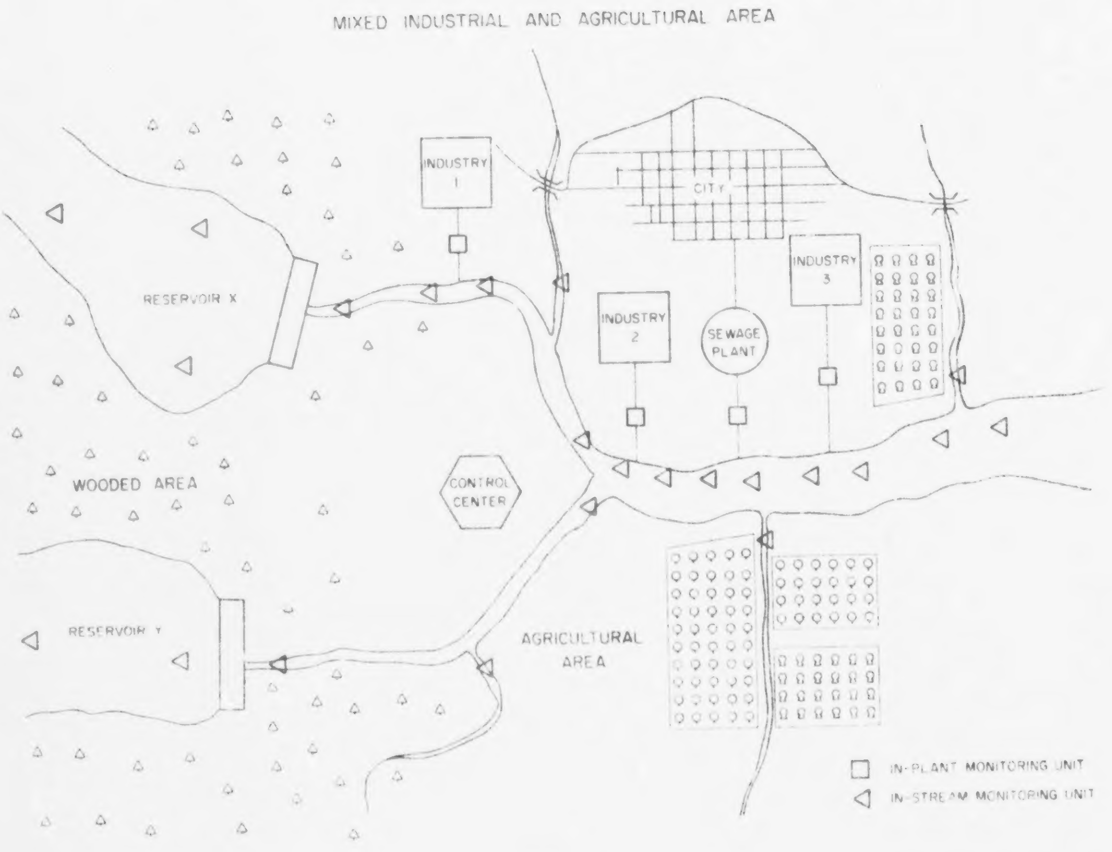
MIXED INDUSTRIAL AND AGRICULTURAL AREA
INDUSTRY 1
CITY
INDUSTRY 3
INDUSTRY 2
SEWAGE PLANT
RESERVOIR X
WOODED AREA
CONTROL CENTER
RESERVOIR Y
AGRICULTURAL AREA
☐ IN-PLANT MONITORING UNIT
◁ IN-STREAM MONITORING UNIT

FIGURE 2
DIAGRAM OF A COORDINATED RIVER BASIN MANAGEMENT SCHEME

D Obviously, the water resources management scheme just outlined is optimistic and depends on the cooperative activities of state and Federal government as well as private users of water resources. However, we are rapidly approaching the time when technology is available to do this job. Implementation of such a program to protect and wisely utilize our water resources now depends on our sincerity.

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This outline was prepared by John Cairns, Jr., Research Professor of Biology and Director, Center for Environmental Studies, and Kenneth L. Dickson, Assistant Professor of Biology and Assistant Director, Center for Environmental Studies, Virginia Polytechnic Institute or State University, Blacksburg, Virginia 24061.

Descriptors:
River Basins, Surveys, Planning, Environment, Balance of Nature, Management

BIOASSAY LABORATORY PROCEDURE

For the bioassay laboratory, the class will be divided into groups. Tests of different substances will be run. Some of the tests will be on an exploratory scale, and one or more will be full scale tests. Both static jar and continuous flow setups may be employed.

General Remarks

Exploratory tests are used for preliminary investigation to determine the general range of toxicity. Usually only two fish, or relatively small numbers of other organisms are used per concentration. For fish 1 to 1-1/2 inches in length, two liters of test solution is usually adequate. These are static jar tests.

Full scale tests are used for intensive study of a restricted range of concentrations, previously determined by exploratory tests. Not less than ten fish or proportionally larger numbers of other organisms are used per concentration. For fish 1 to 2-1/2 inches in length, twenty liters of test solution in two containers should be used for static jar tests (not over 1 gram of fish flesh per liter of water); equipment for continuous flow tests depends on objectives and economics.

Either of the above may be run on a semimicro basis, using a small species of fish (such as guppies) and proportionally smaller quantities of test solution.

Numerous plans have been employed for the use of microscopic organisms on a micro basis.

Class Procedure

Step I

Prepare dilutions of the test material as instructed, using the Dilution Table for reference. Mark containers clearly.

Exploratory scale tests require 2 liters of test solution for each concentration in widemouthed gallon sized jugs.

Full scale tests require 20 liters of solution in 2 widemouthed 5 gallon size jugs, or single larger containers.

Semimicro or micro tests may be set up using smaller quantities of test solution per concentration.

If there is any likelihood that undue quantities of experimental liquid will be needed for chemical analysis, a duplicate series of dilutions should be set up in which no test organisms are placed. As samples are withdrawn from the test containers they can be replaced by equal volumes from this blank series (not necessary in class laboratory exercise).

Step II

Obtain a supply of fish from the stock aquarium and ascertain that they are all in good condition, of approximately the same size, and of the same species.

Introduce two fish into each of the exploratory containers and five (or ten if a single container) into each of the full scale containers, including controls. (Reminder: a complete full scale test should include not less than 10 fish per concentration, preferably in 2 containers.) Note the time on the record sheet. Be sure hands are wet before handling fish, and discard any that get squeezed, fall on floor or bench, or receive any other rough treatment.

Step III

Run a series of chemical analyses on the various dilutions to establish exact conditions at the start of the run. Include DO and other tests such as hardness, pH, etc., as appropriate. Record results on record sheet.

Step IV

Set up petri dishes, each containing a filter paper saturated with formalin in front of each container, to receive dead fish. Mark each petri dish to show the dilution it is to serve.

From time to time as convenient, remove dead fish from test jars and store them in the properly marked petri dishes.

Step V

At the end of each 24 hour period, record on the record sheet for each dilution the total of all fish that have died up to that time including the controls. Likewise check and record levels of DO, pH, and any other changeable parameters being measured.

Step VI

At the end of the test, if significant numbers of control animals have died, serious consideration should be given to whether or not the test results should be used at all. How many are "significant" cannot be arbitrarily defined for all cases, but should certainly not exceed 10%.

If most controls seem to be in good condition, calculate TL_{50}'s for 24 and 96 hours, or for whatever period the test was continued. Record results on record sheet.

BIOASSAY LABORATORY I

I OBJECTIVE

A To practice the fundamentals of setting up bioassay tests.

B To observe the utility of exploratory laboratory tests in setting up full scale tests.

II PROCEDURE

A Work groups: The class will be divided into six work groups designated A to F. These groups will at times work together as "combined groups:" 1(A + B), 2(C + D), and 3(E + F). Each work group should appoint a chairman.

B Each work group will set up one or more exploratory tests according to the following "Organization Plan..."

Follow the general directions given on the "General Bioassay Procedures" sheet.

C Exploratory dilutions should be set up as multiples of ten. That is, 100, 10, 1.0, .1, .01, .001, etc. Each series will consist of 5 dilutions. Each work group should discuss what might be a suitable range for the materials assigned, and then check with the instructor before proceeding.

D Carefully label each container with wax pencil, and make suitable entries on the following "Bioassay Record Sheets." A separate sheet will be needed for each series of containers, and so two sheets are provided, one for the exploratory series, and one for the full scale series.

E No chemical tests will be run in connection with this first laboratory period.

ORGANIZATION PLAN FOR BIOASSAY LABORATORIES

Combined Groups	Work Groups	Exploratory Test Material(s)	Dilution Water	Full Scale Tests
1	A			
	B			
2	C			
	D			
3	E			
	F			

SEC-152
(6-56)
NOTES:
(Dilution waters characteristics, times, series, ect.)

BIOASSAY: Multiple, Exploratory

Plant or Location: ____________ Sheet or Code No.: ____________

Material Tested: ____________ Starting Date: ________ Hour: ________

Source: ____________ Σ Elapsed Hours: _____ Temp: ________

Test Species ____________ No. Inds. per concentration: ________

Remarks: ____________ Technician: ____________

NOTES	Code Numbers	% Survival							
		100 0							
		100 0							
		100 0							
		100 0							
		100 0							

Concentrations in: ____________

SEC-152
(6-56)

BIOASSAY: Multiple, Exploratory

Plant or Location: ____________________
Material Tested: ____________________
Source: ____________________
Test Species: ____________________
Remarks: ____________________

Sheet or Code No. : ____________________
Starting Date: __________ Hour: __________
Σ Elapsed Hours: ______ Temp: ______
No. Inds.per concentration: __________
Technician: ____________________

NOTES: (Dilution waters characteristics, times, series, ect.)	Code Numbers	% Survival							
		100 0							
		100 0							
		100 0							
		100 0							
		100 0							

Concentrations in: ______________________________

BIOASSAY LABORATORY II

I OBJECTIVES

A To evaluate results of Exploratory Tests

B To set Up Full Scale Tests

C To Institute a Series of Suitable Chemical Analyses

II PROCEDURE

A Combination of Groups

Groups will combine as follows: A + B, C + D, E + F. Each combined group will now agree as to which of the exploratory tests will be extended to a full scale test.

B Set Up Full Scale Tests

1 The range tested will be indicated by the exploratory test. It should extend from the lower endpoint, so selected that all individuals are reasonably sure to survive, to 10X that concentration. Consult with the instructor before finally setting up.

2 Use the intervals between concentrations as given in Table 1 of the lecture "Fundamentals of the Toxicity Bioassay," and also on the "TL__ Paper" (which follows).

3 Set up a "Bioassay Record Sheet."

C Make appropriate observations and record at 24, 48, and 96 hour intervals.

III Calculate TL_{50}'s for each period of time for each test.

IV CHEMICAL TESTS

In order to evaluate the chemical factors which influence toxicity, and to determine the effect of the test materials on the chemical composition of the solutions, selected chemical tests should be performed routinely. Since it can be usually assumed that interaction will be greater at the higher concentrations, run each chemical test on the highest concentration, and also on an intermediate concentration if time permits. Compare in all cases with the control.

A Dissolved Oxygen

Determine the DO of selected concentrations of test solutions as indicated above. Difficulties in accurate measurement may be encountered in paper mill wastes and detergent solutions.

B Alkalinity and Hardness

Determining the total alkalinity and total hardness. If the pH (see below) of metal solutions are less than 7.0, report alkalinity.

C pH

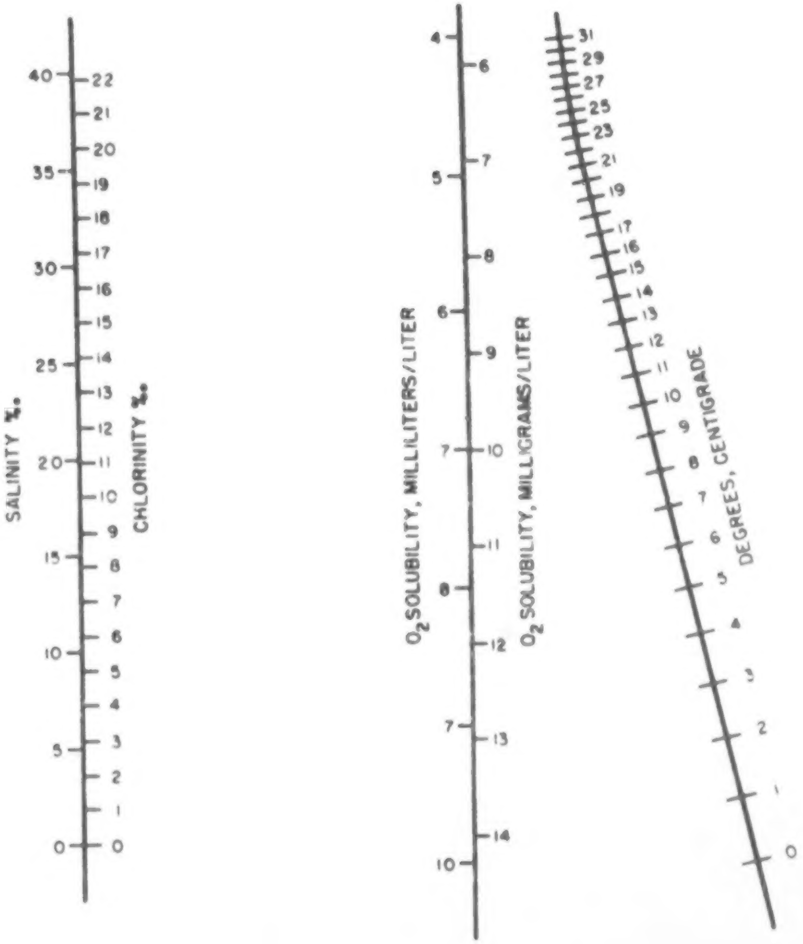
Determine the pH, using the glass electrode.

D For tests involving seawater, determine the salinity by the method(s) specified. Refer to the Nomogram for the Solubility of Oxygen.

DILUTION TABLE

Concentration desired			To prepare solutions of concentration indicated at left, take number of milliliters of stock solution shown below, and make up to one liter with suitable dilution water.					
%	ppm or mg/l	ppb or µg/l	Stock sol: 1% 10 gm/l	Stock sol: .1% 1 gm/l	Stock sol: .01% 1 gm/l	Stock sol: .001% .01 gm/l	Stock sol: .0001% .001 gm/l	Stock sol: .00001% .0001 gm/l
100.	1,000,000							
10.	100,000							
1.0	10,000		1000					
.56	5,600		560					
.32	3,200		320					
.18	1,800		180					
.1	1,000		100	1000				
.056	560		56	560				
.032	320		32	320				
.018	180		18	180				
.01	100		10	100	1000			
.0056	56		5.6	56	560			
.0032	32		3.2	32	320			
.0018	18		1.8	18	180			
.001	10		1.0	10	100	1000		
.00056	5.6			5.6	56	560		
.00032	3.2			3.2	32	320		
.00018	1.8			1.8	18	180		
.0001	1.0	1000		1.0	10	100	1000	
.000056	.56	560			5.6	56	560	
.000032	.32	320			3.2	32	320	
.000018	.18	180			1.8	18	180	
.00001	.10	100			1.0	10	100	1000
.0000056	.056	56				5.6	56	560
.0000032	.032	32				3.2	32	320
.0000018	.018	18				1.8	18	180
.000001	.010	10				1.0	10	100
.00000056	.0056	5.6					5.6	56
.00000032	.0032	3.2					3.2	32
.00000018	.0018	1.8					1.8	18
.0000001	.0010	1.0					1.0	10
.000000056	.00056	.56						5.6
.000000032	.00032	.32						3.2
.000000018	.00018	.18						1.8
.00000001	.0001	.10						1.0

Originally prepared for Training by C. Henderson; modified and arranged by H. W. Jackson.



NOMOGRAM FOR THE SOLUBILITY OF OXYGEN

RICHARDS AND CORWIN, LIMNOLOGY AND OCEANOGRAPHY, 1956

SEC 151
(6-58)

BIOASSAY RECORD SHEET

Series: Company: Date:

Technician: Starting Hour

Material being tested:

Source:

Source of dilution water:

Test species Temp. range:

No. individuals per concentration:

Start

Dilution:									Control
DO									
pH									
Hardness									
Other									

24 hours

No surviving									
% survival									
DO									
pH									
Other									

48 hours

No surviving									
% survival									
DO									
pH									
Other									

96 hours

No surviving									
% survival									
DO									
pH									
Other									

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USE OF TL__ PAPER

NOTE: The "TL__ Paper" has been especially designed for use in the Training Program of Water Programs Operations. (It is not known to be commercially available.) The same results can be obtained with conventional graph paper (two-cycle semi-log is recommended).

I INTRODUCTION

A Measurements of Toxicity

Bioassay results using fish are expressed in terms of Tolerance Limits (TL) for time "t". The percentage of experimental animals surviving for the specified period of time is written as a subscript to the TL symbol. For example, the "96-hour TL_{50}" is that concentration of a substance which 50% of the experimental fish can tolerate for 96 hours. The TL_{50} is equivalent to the median tolerance limit (TL_m).

The use of TL with the percentage subscript allows the designation of percentage survivals other than 50%; e.g., a TL_{10}^{t} would indicate that only 10% of the fish could tolerate a given concentration for time t, while a TL_{90}^{t} would indicate a concentration which could be tolerated by 90% of the fish for the time specified. The TL_{50} is currently the standard and should always be determined.

Unless specified to the contrary, the laboratory exercise in this training course will concentrate on the determination of TL_{50}'s, and the instructions below are so written.

B Preliminary Procedures

Examine the "control" container. A bioassay test should not be accepted as reliable unless at least 90% of the control animals survive. Death of any of the controls should be clearly explained in the "Notes" at the right. If control survival is satisfactory, proceed as follows (if not, repeat the test).

II PREPARATIONS FOR CALCULATING A TL_{50}

A Fill in preliminary information as called for on the right side of the sheet, including the subscript "50" in the title and also in the box after "Final Results"; and the time intervals to be employed; e.g., 15 min., 1 hr., 4 hrs., or 24 hrs., 48 hrs., 96 hrs., etc. (These are the Time "t's"). Circle the term in which the experimental concentrations are expressed; fill in the name of the test species, the temperature range, and describe the dilution water. Any number of TL_m's may be calculated from a given setup at successive time intervals.

B Insert decimal points and/or zeros in the column of numerals above "Bioassay Concentrations" to represent the dilutions actually used in the test. If the series used does not fit the lines provided, use the coordinate TL__ Paper, or request further instructions.

III TO ESTIMATE THE TL_{50} AT TIME "T"

A Find the "Percent Survival" scale at the bottom of the graph. Indicate the percent survival at each of the concentrations tested. Use a code to mark the points at successive times as: a tiny circle, a triangle, a square, or a color code.

B Locate the highest test concentration showing greater than 50% survival. Connect this point to the survival percentage of the next highest concentration with a straight line.

USE OF TL__ PAPER

NOTE: The "TL__ Paper" has been especially designed for use in the Training Program of Water Programs Operations. (It is not known to be commercially available.) The same results can be obtained with conventional graph paper (two-cycle semi-log is recommended).

I INTRODUCTION

A Measurements of Toxicity

Bioassay results using fish are expressed in terms of Tolerance Limits (TL) for time "t". The percentage of experimental animals surviving for the specified period of time is written as a subscript to the TL symbol. For example, the "96-hour TL_{50}" is that concentration of a substance which 50% of the experimental fish can tolerate for 96 hours. The TL_{50} is equivalent to the median tolerance limit (TL_m).

The use of TL with the percentage subscript allows the designation of percentage survivals other than 50%; e.g., a TL_{10}^{t} would indicate that only 10% of the fish could tolerate a given concentration for time t, while a TL_{90}^{t} would indicate a concentration which could be tolerated by 90% of the fish for the time specified. The TL_{50} is currently the standard and should always be determined.

Unless specified to the contrary, the laboratory exercise in this training course will concentrate on the determination of TL_{50}'s, and the instructions below are so written.

B Preliminary Procedures

Examine the "control" container. A bioassay test should not be accepted as reliable unless at least 90% of the control animals survive. Death of any of the controls should be clearly explained in the "Notes" at the right. If control survival is satisfactory, proceed as follows (if not, repeat the test).

II PREPARATIONS FOR CALCULATING A TL_{50}

A Fill in preliminary information as called for on the right side of the sheet, including the subscript "50" in the title and also in the box after "Final Results"; and the time intervals to be employed; e.g., 15 min., 1 hr., 4 hrs., or 24 hrs., 48 hrs., 96 hrs., etc. (These are the Time "t's"). Circle the term in which the experimental concentrations are expressed; fill in the name of the test species, the temperature range, and describe the dilution water. Any number of TL_m's may be calculated from a given setup at successive time intervals.

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III TO ESTIMATE THE TL_{50} AT TIME "T"

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B Locate the highest test concentration showing greater than 50% survival. Connect this point to the survival percentage of the next highest concentration with a straight line.

C Read on the scale at the left, the value of the point where the above line and the 50% survival line intersect. This value is the TL_{50} concentration for the time interval in question.

D If there are points below (i.e., at lesser concentrations) which show less than 50% survival, an unreliable population of experimental animals, poor handling, or other detrimental factor may be indicated. Re-examine the survival of the controls. If it is less than 100%, consider the advisability of repeating the test.

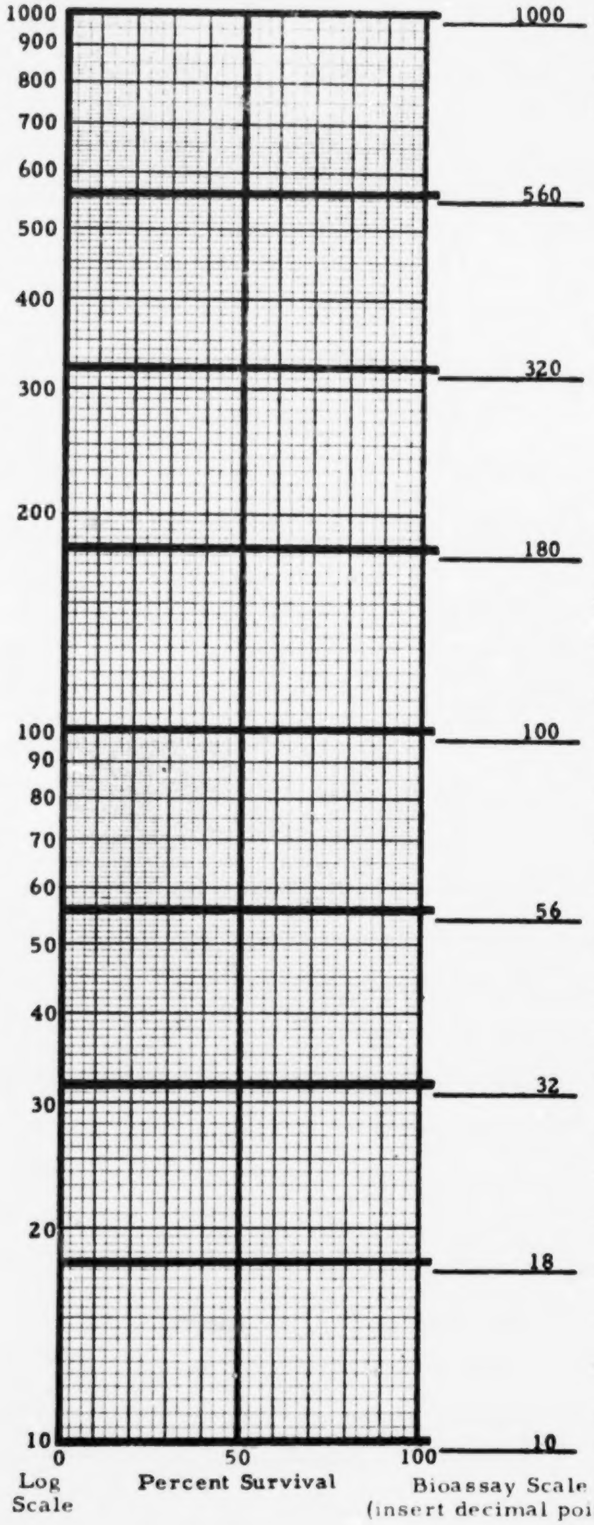
IV COMMENTARY

The TL_{50}^{t} concentration is that which will permit half of the experimental organisms to survive for time "t" under the conditions of the test. If by chance one of the experimental concentrations happened to have 50% survival at time "t" that is the TL_{50} concentration, no further calculation is necessary (provided there is no higher concentration which showed an equal or higher survival rate).

This outline was prepared by H. W. Jackson, Chief Biologist, National Training Center, WPO, EPA, Cincinnati, OH 45268.

TL PAPER

Sheet No.or Code:________



Material Tested:____________________

Starting Date: Hour:

Final Results:

	Time	Intervals		
TL				

Concentrations Expressed as (circle one):

%, mg/l, other:____________

Test species:____________

Temperature Range:____________

Dilution Water(source & characteristics):

Notes:____________

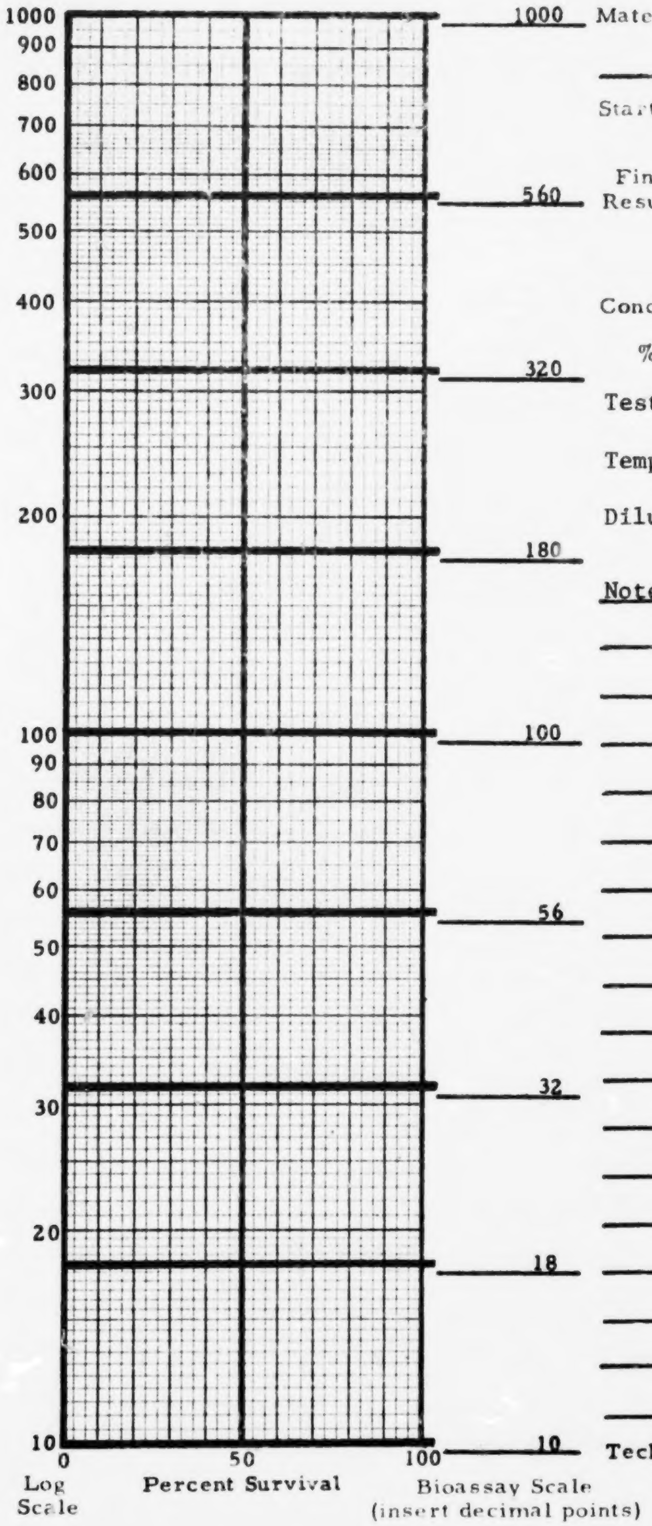
Technician:____________

Note: This paper not commercially available.

BI. BIO. met.15b.6.73

TL PAPER

Sheet No.or Code:



Material Tested:

Starting Date: Hour:

Final Results:

	Time Intervals			
TL				

Concentrations Expressed as (circle one):

%, mg/l, other:

Test species:

Temperature Range:

Dilution Water(source & characteristics):

Notes:

Technician:

Note: This paper not commercially available.

BI. BIO. met.15b.6.73

SEC 187
(Rev. 11-69)

TL$_m$ PAPER
(Coordinate)

Sheet No. or Code: ______

Material Tested: ______

______

Starting Date: Hour:

Final Results:

TL	Time Intervals		

Concentrations Expressed as (circle one):

%, mg/l, other: ______

Test species: ______

Temperature Range: ______

Dilution Water (source & characteristics):

______

Notes: ______

Technician: ______

0 50 100

Scale Percent Survival Bioassay Concentrations

Note: This paper not commercially available: BI.BIO.met.16a.11.69

SEC 187
(Rev. 11-69)

TL_m PAPER
(Coordinate)

Sheet No. or Code: ____

Material Tested: ____

____

Starting Date: Hour:

Final Results:

TL	Time Intervals		

Concentrations Expressed as (circle one):

%, mg/l, other: ____

Test species: ____

Temperature Range: ____

Dilution Water (source & characteristics):

____

Notes: ____

Technician: ____

0 50 100

Scale Percent Survival Bioassay Concentrations

Note: This paper not commercially available:

BI.BIO.met.16a.11.69

SEC 151
(6-58)

BIOASSAY RECORD SHEET

Series: ______________ Company: ______________________________ Date: ________

Technician: ______________________________ Starting Hour ________

Material being tested: ______________________________

Source: ______________________________

Source of dilution water: ______________________________

Test species ______________________________ Temp. range: ________

No. individuals per concentration: ______________________________

	Start								
Dilution:									Control
DO									
pH									
Hardness									
Other									
	24 hours								
No surviving									
% survival									
DO									
pH									
Other									
	48 hours								
No surviving									
% survival									
DO									
pH									
Other									
	96 hours								
No surviving									
% survival									
DO									
pH									
Other									

BI. BIO. met. 9c. 11.69

SEC 151
(6-58)

BIOASSAY RECORD SHEET

Series: ______________ Company: ______________________________ Date: ________

Technician: ______________________________________ Starting Hour ________

Material being tested: ____

Source: ____

Source of dilution water: ____

Test species ______________________________________ Temp. range: ________

No. individuals per concentration: ___________________________________

	Start								
Dilution:									Control
DO									
pH									
Hardness									
Other									

	24 hours								
No surviving									
% survival									
DO									
pH									
Other									

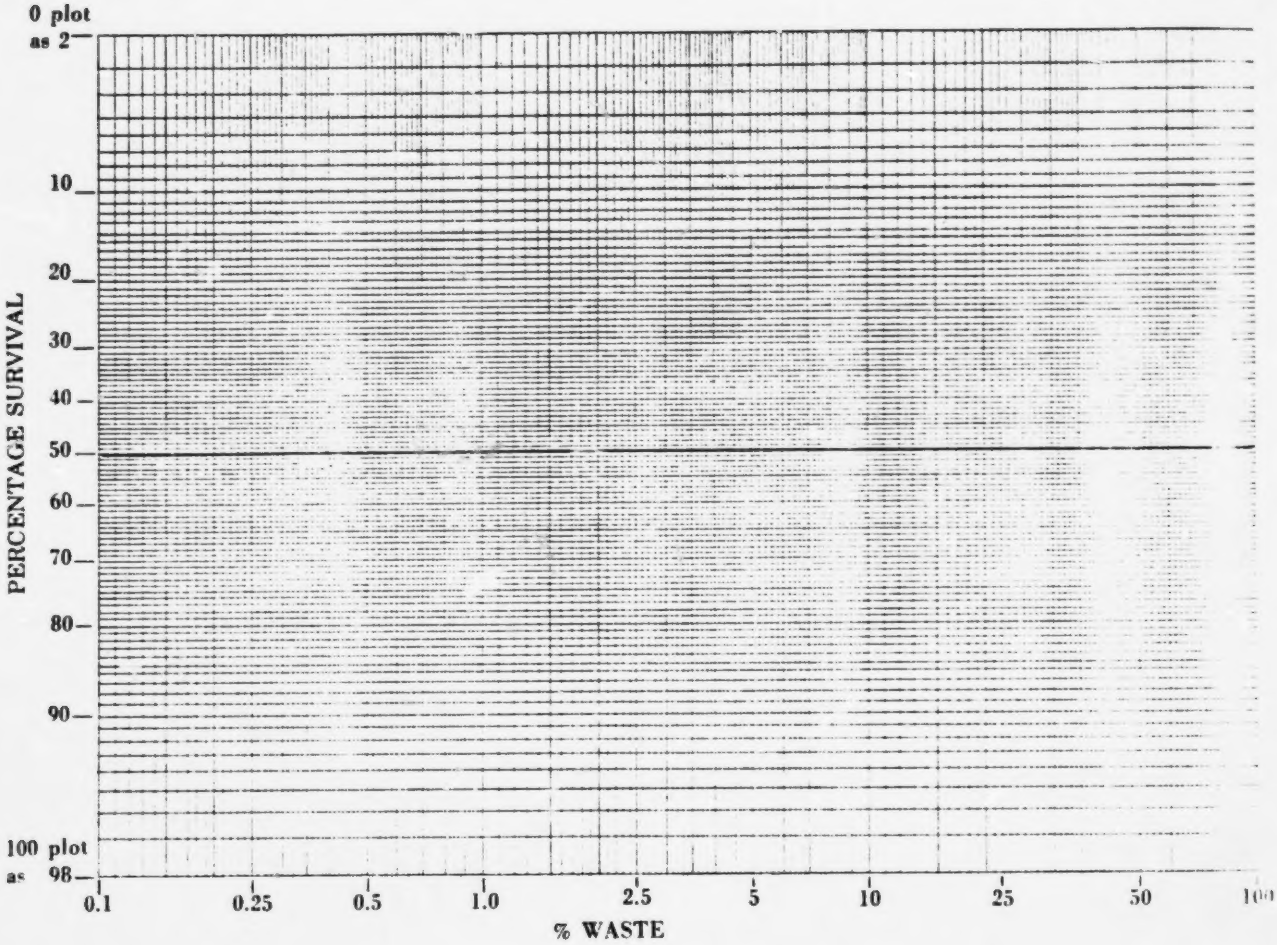
	48 hours								
No surviving									
% survival									
DO									
pH									
Other									

	96 hours								
No surviving									
% survival									
DO									
pH									
Other									

BI. BIO. met. 9c. 11.69

(Extracted from: ORSANCO 24-Hour Bioassay, January 1974)

BIOASSAY PAPER **(Log-probit)** **Code:**________



Material Tested:________

Starting Date:________ **Hour:**________

Concentrations expressed as (circle one): %, mg/liter.________

Test species:________ **Temperature:**________

Dilution water source and characteristics:________

Other notes:________

Final Results:

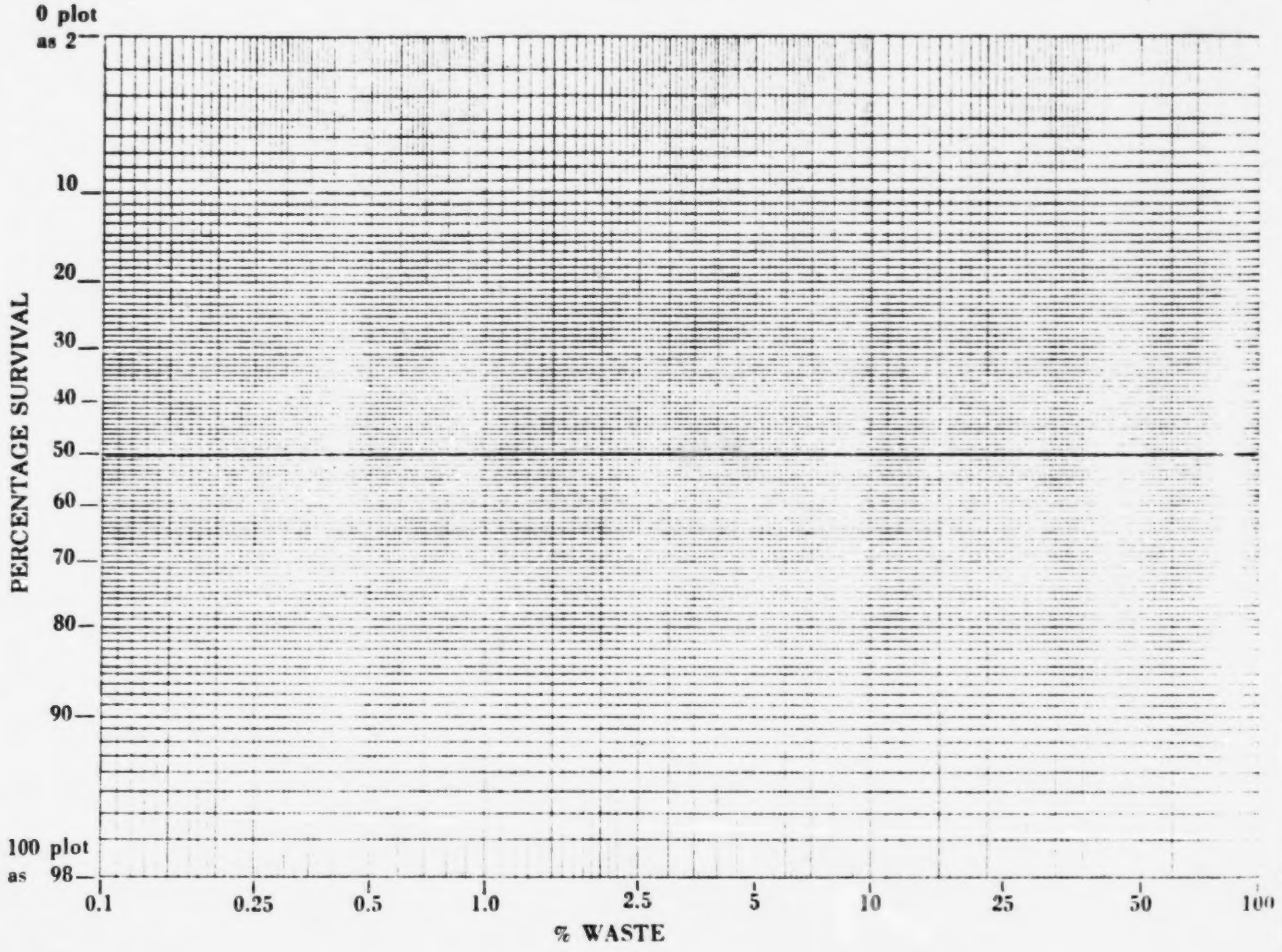
Time interval		24 hr.	48 hr.	96 hr.	
LC50					

Observer:________

BI. AQ. 32. 11. 74

(Extracted from: ORSANCO 24-Hour Bioassay, January 1974)

BIOASSAY PAPER **(Log-probit)** **Code:**



Material Tested:

Starting Date: **Hour:**

Concentrations expressed as (circle one): %, mg/liter,

Test species: **Temperature:**

Dilution water source and characteristics:

Other notes:

Final Results:

Time interval		24 hr.	48 hr.	96 hr.	
LC50					

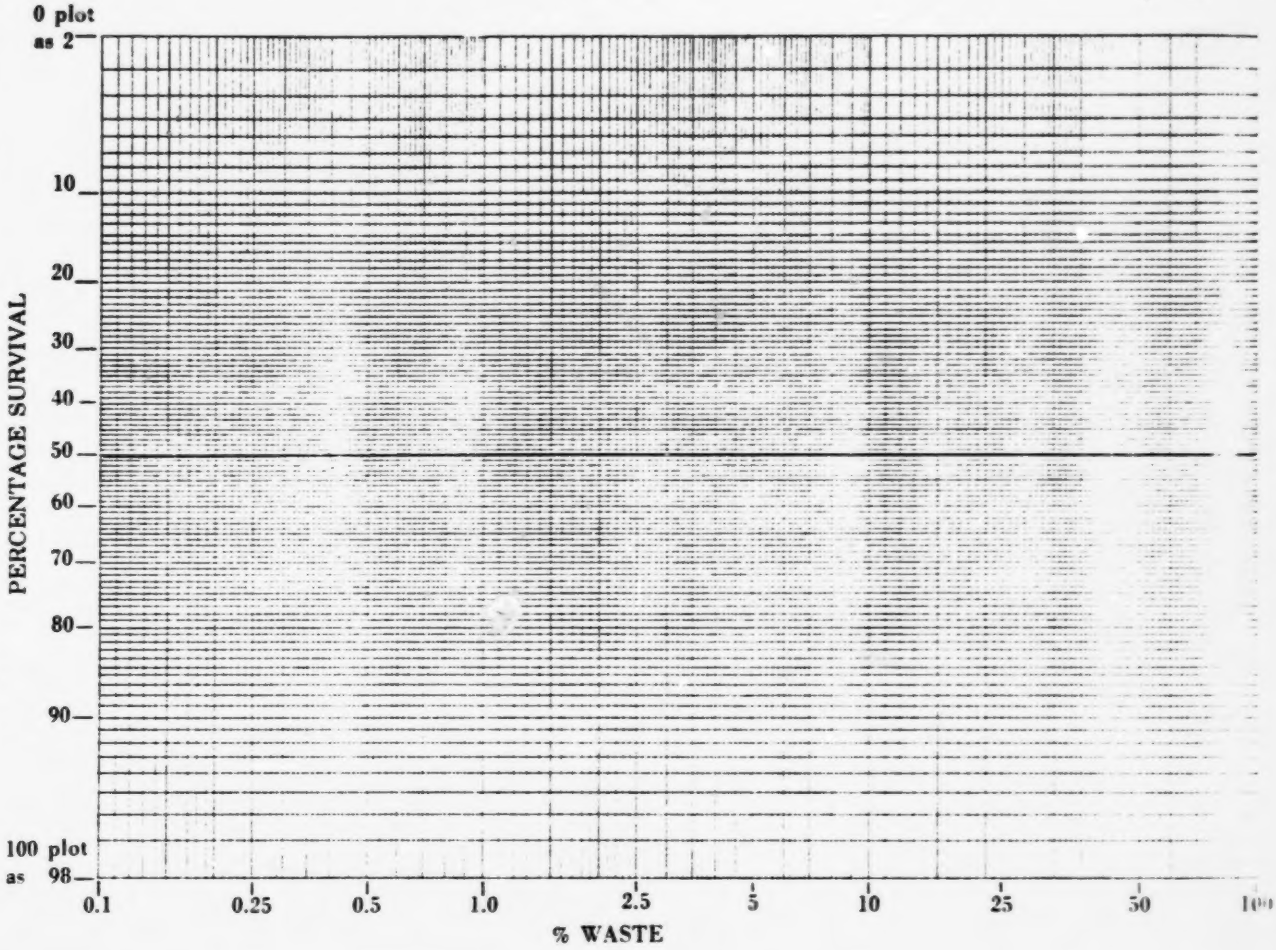
Observer:

BI. AQ. 32. 11. 74

(Extracted from: ORSANCO 24-Hour Bioassay, January 1974)

BIOASSAY PAPER (Log-probit)

Code:



Material Tested:

Starting Date: Hour:

Concentrations expressed as (circle one): %, mg/liter.

Test species: Temperature:

Dilution water source and characteristics:

Other notes:

Final Results:

Time interval		24 hr.	48 hr.	96 hr.	
LC50					

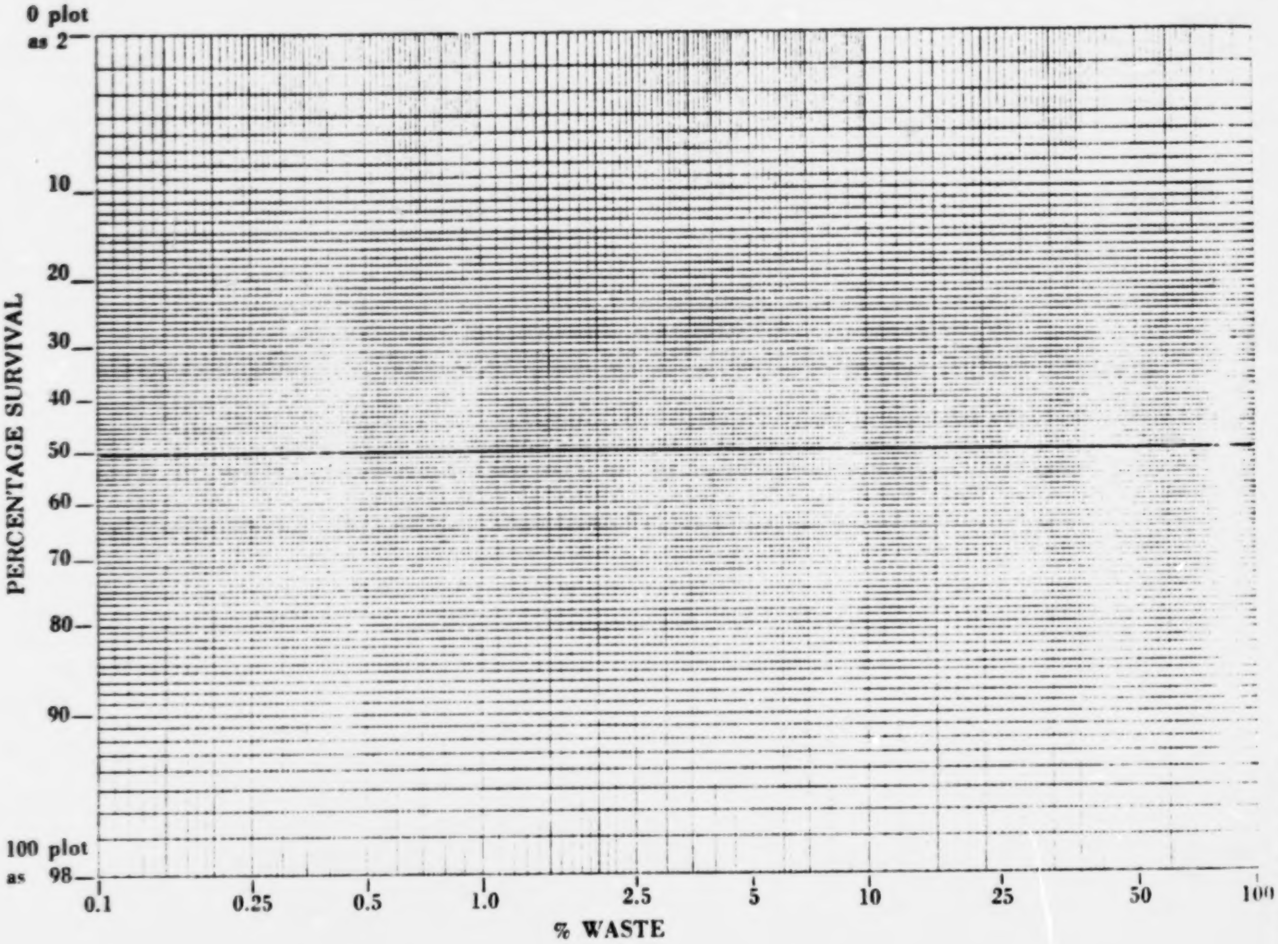
Observer:

BI. AQ. 32. 11. 74

(Extracted from: ORSANCO 24-Hour Bioassay, January 1974)

BIOASSAY PAPER (Log-probit)

Code: ____________



Material Tested: ____________

Starting Date: ____________ **Hour:** ____________

Concentrations expressed as (circle one): %, mg/liter ____________

Test species: ____________ **Temperature:** ____________

Dilution water source and characteristics: ____________

Other notes: ____________

Final	Time interval		24 hr.	48 hr.	96 hr.	
Results:	LC50					

Observer: ____________

BI. AQ. 32. 11. 74